Comparison of stool antigen assay (HpSA) and urea breath test (UBT) in detecting Helicobacter pylori

Asia Pacific Journal of Allied Health Sciences
Vol. 4, No 1, pp. 38-45
December 2021
ISSN 2704-3568

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Abstract – *Helicobacter pylori is spiral-shaped bacterium that grows in the digestive tract and tends to attack the stomach lining. It causes gastritis and peptic ulcers and is associated with the development of gastric cancer. Approximately 50% of the world population is infected with H. pylori with the highest prevalence rates in developing countries. In the Kingdom of Saudi Arabia, many people need to be tested for H. pylori. The participants (104) of the study, who were mostly females (66) with age range of 15-76 years old, were from the gastrointestinal clinic and were screened using Anti-Helicobacter pylori (ELISA) IgG. Stool antigen assay was also performed, the results of which were compared with the Urea Breath Test (UBT). Using McNemar test, the results obtained between ELISA IgG and HpSA and UBT, were found to be significantly different (p=0.000), proving that the two diagnostic procedures are better than ELISA in providing accurate results. On the other hand, there was no significant difference between HpSA and UBT which shows that they are comparable. Thus, HpSA can be used as an alternative confirmatory test in detecting H. pylori.*

Keywords – *Helicobacter pylori, stool antigen assay, urea breath test*

INTRODUCTION

Helicobacter pylori is a gram-negative spirally shaped bacterium that exclusively exists in the mucosa of the stomach, particularly in the antrum. It is adapted to live in the harsh, acidic environment of the stomach and has the capacity to reduce its acidity for its survival. Its shape allows it to penetrate the stomach lining; thus, it becomes protected by mucus where the body's immune cells could not reach it. This bacterium can interfere with the immune response and ensure that it will not be destroyed which can lead to stomach problems. H. pylori normally infects the stomach during childhood [1]. Meuler [2] revealed that the bacterium was present almost exclusively in patients with chronic gastritis and was also common in those with peptic ulceration of the stomach or duodenum. The correlation between the colonization of the stomach by H. pylori and gastric lymphoma has been demonstrated in multiple studies [3].

Helicobacter pylori infection is well known to be the most common human infection worldwide on the basis of the fact that approximately 50% of the world's populations are infected and that human beings are the main reservoir [3]. Infections are usually harmless, but H. pylori is responsible for the majority of ulcers in the stomach and small intestine. While infections with this strain of bacteria typically does not cause symptoms, they can lead to diseases in some people, including peptic ulcers and inflammatory condition inside the stomach known as gastritis and has been associated with several serious diseases of the gastrointestinal tract, including duodenal ulcer and gastric problems which in turn can lead to cancer [1] [2]. Since its discovery in 1983 by Warren and Marshall, H. pylori has been the topic of extensive research [2].

The pattern of infection in an early childhood acquisition of H. pylori is 30%-50% that reaches over 90% during adulthood in developing countries. Infection in developed countries is less common in young children and reaches up to 60% in older ages [4]. In the vast majority of individuals, infection is acquired during childhood with those of low socioeconomic means and having infected family members being at highest risk for early childhood acquisition. Definitive routes of transmission of the infection are unclear, with evidence suggesting oral-oral, gastric-oral, and fecal-oral routes. If untreated, H. pylori infection is lifelong. Although clinical disease typically occurs decades after initial infection acquisition, children infected with H. pylori may have gastritis, ulcers, mucosal-associated lymphoid type lymphoma, and, rarely, gastric atrophy with or without intestinal metaplasia that are both precursor lesions for gastric cancer [5]. In 1994, the International Agency for Research on cancer classified H. pylori as a class I carcinogen in humans. It causes

chronic active gastritis, duodenal and gastric malignancy, and is thought to be associated with coronary artery disease, cerebral stroke, vitamin B12 and iron-deficiency anemia. According to Pathak, Bhasin, and Khanduja [6] noninvasive test and treatment strategies are widely recommended in primary settings.

H. pylori prevalence is highly variable in relation to geography, ethnicity, age and socioeconomic factors. It is high in developing countries and lower in the developed world [7]. Several studies have shown that the prevalence of H. pylori is still high in most countries. In North European and North American populations, about one-third of adults are still infected, whereas in South and East Europe, South America and Asia, the prevalence of H. pylori is often higher than 50%. H. pylori remain highly prevalent in immigrants coming from countries with high prevalence of H. pylori [8].

In the Kingdom of Saudi Arabia (KSA), the prevalence of H. pylori infection is about 80% of the adult population [7]. The high prevalence is a public health issue that requires public health interventions. Peptic ulcer disease and gastric carcinoma are common in KSA and as in many populations, they account for major health care cost and significant economic loss from absenteeism, morbidity and deaths from complications. Reports, mainly from urban populations indicated a relatively high prevalence of H. pylori infection in KSA which suggests differences in the prevalence between urbanized and rural population [9]. Interpersonal transmission appears to be the main route, although environmental transmission, such as drinking contaminated water, remains possible. Transmission events were more frequent between close relatives and between individuals living in the same house [8]. The principal reasons for these variations involve socioeconomic differences between populations. Transmission of H. pylori is largely by the oral-oral or fecal-oral routes. Food prepared under less than ideal conditions or exposed to contaminated water or soil may increase the risk. Inadequate sanitation practices, low social class, crowded or high-density living conditions seem to be related to a higher prevalence of H. pylori infection. Poor hygiene and crowded conditions may facilitate transmission of infection among family members [9].

Diagnostic tests for H. pylori infection include endoscopic and non-endoscopic methods. The techniques used may be direct methods like rapid urea test (RUT), histology, culture, fluorescence in situ hybridization (FISH), polymerase chain reaction (RCR), or indirect methods using stool antigen (SAT), finger stick serology test, whole blood serology, 13C or 14C urea breath test [7]. The tests used can also be classified as noninvasive and invasive. Noninvasive tests, such as the urea breath test, stool tests, and blood tests, like whole blood and serologic assays, detect the presence or absence of infection. On the other hand, invasive tests, like upper gastrointestinal endoscopy with gastric biopsy, can determine both the presence or absence of infection and the extent and severity of mucosal injury [5].

Rapid urease test has the highest sensitivity and specificity but it is not readily available in all parts of the world, it is rapid and cheap but the post-treatment sensitivity is reduced. In histology, the detection is improved by using special stains like the Warthin-Starry silver stain or the cheaper hematoxylin-eosin (H&E) stain or Giemsa staining protocol. Furthermore, culture is highly specific but has poor sensitivity if adequate transport media are not available. It requires experience or expertise and expensive; thus, may not be practical in all countries. PCR is sensitive and specific, but it is not standardized and considered experimental. Finger stick serology test is very poor and cannot be equated with ELISA serology [7].

Enzyme-linked immunosorbent assay (ELISA) serology is less accurate and does not identify active infection. It is a reliable predictor of infection in high prevalence developing countries, but is not recommended after therapy although it is cheap and readily available. Antibodies against H. pylori occur in about 70% of patients with chronic active gastritis, and are associated with ulcer conditions in 60-90% of cases. After contact with H. pylori, IgA, IgG and IgM antibodies against H. pylori can occur in the serum. The specific IgM disappears after a few weeks. IgA antibodies can still be detected after a considerable period Elevated IgG titers are frequently found after the IgM titer has fallen and can persist over many years. IgA antibodies are formed locally, but are not detected in the serum in every case. A positive IgA result correlates well with gastritis activity. An elevated IgG antibody titer is considered to be a marker for chronic infection. Test for specific IgG antibodies against H. pylori is a suitable indicator for the complete eradication of the agent as a part of therapy monitoring. A significant drop in the IgG antibody titer after about 6 weeks of therapy is a sign of success [11].

13C or 14C urea breath test (UBT) is recommended for diagnosis of H. pylori before

treatment but the availability is variable. It is the preferred test for confirming eradication but must not to be performed within 2 weeks of proton pump inhibitor (PPI) therapy or within 4 weeks of antibiotic therapy [10]. UBT is recommended by European H. pylori experts as the first choice and the most reliable non-invasive test for diagnosing an active infection as well as follow-up of eradication of infection [12]. It is the most recommended non-invasive method for monitoring the outcome of eradication therapy in patients who do not need repeat endoscopy [13]. It is also a commonly used non-invasive test to diagnose H. pylori in patients with dyspepsia with a high diagnostic accuracy. UBT using either 13C or 14C provides a noninvasive diagnostic method for the detection of active H. pylori infection. The ^{14}C-Urea doze is very low (1 μCi), this corresponds to a body exposure of 0.0006 mSi, which is less than an ordinary x-ray [12]. However, 13C urea breath test has an advantage over the 14C version, because the 13C isotope is a nonradioactive natural isotope; therefore a user's license is unnecessary, making the handling and mailing of samples simple. The 13C UBT test is preferred in children and expectant mothers. UBT is indicated to confirm H. pylori colonization and monitor its eradication [14]. Bacteria other than H. pylori that produce urease are rarely found in the gastric flora. The presence or absence of a H. pylori infection can thus be determined by detecting if labeled CO_2 is present in exhaled air shortly after intake of labeled urea [12]. Moreso, 14C UBT is a reliable indicator of H. pylori eradication after treatment. It can obviate the need for antral biopsies to confirm eradication of H. pylori after completion of treatment [15]. Logan [16] and Manes et al. [17] concluded that UBT is a very accurate test for detecting H. pylori infection with sensitivity and specificity better than many other tests for H. pylori. UBT detects much lower levels of H. pylori infection and by assessing the entire gastric mucosa. UBT is a reliable indicator of H. pylori eradication after treatment [15]. In view of its high sensitivity and specificity, the UBT is useful for rapid, non-invasive diagnosis of H. pylori infection [18].

Therefore, UBT remains the first-line non-invasive test in the assessment of the presence of H. pylori infection. HpSA should be used only when UBT is not available, as also recommended by the H. pylori experts participating in the Maastricht 2-20000 Consensus Conference [13].

Stool antigen assay (HpSA) is not often used in spite of its high sensitivity and specificity before and after treatment. There should have a more prominent place, as it is inexpensive and non-invasive. The immunochromatographic technique (rapid) for the detection of H. pylori antigen has substantially resolved these problems, ensuring a serological monitoring in a very short space of time using simple, highly specific technology without recourse to invasive techniques. The fecal test for H. pylori antigen can be utilized as a rapid screening process for large populations of patients and highly indicated in the early diagnosis of H. pylori infection as the immune response can often precede clinical manifestations of disease. From a diagnostic point of view, a high level of H. pylori antigen must be interpreted as an indication of type B asymptomatic gastritis [19].

ImmunoCard HpSA STAT is a simple noninvasive and accurate test for the diagnosis of H. pylori infection (Li, Gou, Zhang, Zhao, & Da, 2004). It is a rapid, simple and accurate in-clinic test for pre-eradication diagnosis of H. pylori and post-eradication follow-up [20].

The choice of test depends to a large extent on availability and cost, and includes a distinction between tests used to establish a diagnosis of infection and those used to confirm its eradication. Other important factors includes the clinical situation, population prevalence of infection, pretest probability of infection, differences in test performance and factors that may influence the test results such as the use of anti-secretory treatment and antibiotics [7].

Most of the physicians in KSA request ELISA IgG in the screening of the infection, but this will not distinguish previous exposure and colonization from current infection of the patient (She, Wilson, & Litwin, 2009). In order for them to determine the status of the present condition of the patient, another diagnostic test is performed. The most common confirmatory diagnostic test used in KSA is 14C UBT. It is widely available but the material is radioactive; thus, may pose harm. Even though the dose is safe and very small, the radioactivity makes this a less attractive test for follow-up and repeats [21]. The non-invasive and safer method using 13C UBT is not yet available because of the relatively expensive isotope ratio mass spectrophotometer needed to perform it [22].

Stool antigen test (HpSA) is simple, cheap and non-invasive method for accurate diagnosis of H. pylori infection [23]. In this test, the patient preparation and sample collection is very convenient.

Due to high prevalence of H. pylori infection in Saudi Arabia, many people need to be tested and

undergo with the different diagnostic procedures in detecting the infection. Initial serology testing is practical and affordable but follow-up testing like urea breath or stool antigen test is recommended for patients with a history of ulcer complications or cancer and to determine cure, if indicated [2]. Since UBT cannot be performed in children and pregnant women due to the use of 14C capsule that contains radioactive material; and the test is expensive, the study was conducted to determine if stool antigen assay (HpSA) is comparable with UBT in the detection of Helicobacter pylori. Specifically, the study aims to determine the ELISA IgG, UBT and HpSA results and identify the significant difference in the results obtained in HpSA and UBT in detecting H. pylori. If HpSA will be proven to be comparable with UBT, then this study will help the requesting physician to select a non-invasive, safer and cheaper method for H. pylori detection. The use of HpSA will also make the testing more affordable for patients who will be required to undergo H. pylori detection.

MATERIALS AND METHODS

Participants

A total of 104 participants who came from the gastrointestinal tract clinic (GIT) of Dr. Sulaiman Al Habib Medical Group- Al Takhassusi Hospital, Kingdom of Saudi Arabia were included in the study. Clinically submitted specimens were used with the approval from the Laboratory Director and consent letter was sought.

Exclusions and Inclusions

The patients at the gastrointestinal clinic who were required to undergo the three diagnostic procedures, namely anti-Helicobacter ELISA IgG, HpSA, and UBT regardless of their clinical conditions were included as participants in the study. Those who were tested with two methods were excluded.

Sample Analysis

A. Anti- *Helicobacter pylori* ELISA (IgG)

Serum was collected from the participants and samples were examined using EUROIMMUN analyzer. All reagents were brought to room temperature (18°C to 25°C) prior to use.

Coated wells were loaded to the microplate wells. Wash buffers and other consumables were checked to ensure that the volume was enough to perform the test. All reagents were loaded including the enzyme conjugate that was thoroughly mixed, the substrate and stop solution through barcode readers. After loading the reagents, calibrators 1, 2 and 3 were loaded along with the positive and negative controls including the serum of the participants. Specific *H. pylori* IgG icon in the machine will be pressed for sample processing. After 2 hours and 30 minutes, results were produced by the machine and were ready for printing. The validity of the results is dependent on the acceptable results of the calibrators and controls. The results were interpreted as negative when the obtained values are <16 RU/mL, borderline for ≥16-<22 RU/mL, and positive for ≥22 RU/Ml [11].

Figure 1. EUROIMMUN Analyzer

B. Urea Breath Test

The participants were advised to fast for at least 4-6 hours, preferably overnight. For those who were in medications, they were advised not to take antibacterial or bismuth medication therapy at least 4 weeks, proton inhibitor for at least 1 week, and H2-receptor antagonist, antacid or sucrulfate for at least 24 hours. They were given a capsule of ^{14}C labeled urea with only small amount of water. After 10 minutes, they were instructed to blow the breath card until the color changed from orange to yellow. Then, the card was measured in the Heliprobe™ Analyzer [7]. A negative or positive infection is presented immediately on the LCD. The cut-off levels have a low level and a high level, count per minute (cpm) value below the low level is defined as a negative result (Heliprobe 0) which is <50 cpm and value above or equal to the high level is defined as a positive result (Heliprobe 2) which is ≥50 cpm [7].

Figure 2. Urea Breath Test Heliprobe Analyzer

C. Stool antigen assay (HpSA)

The participants were instructed to collect stool samples in a container without media or preservative and submit such in the clinical laboratory. Test devices and extracted samples were allowed at room temperature prior to testing using One step *H. pylori* antigen in human fecal specimen (HpSA) [19]. Assay diluents were filled up to the Fill Line and were transferred into the sample collection tube twice. By using the sample collection swab, 50 mg of fecal material was collected by inserting the sterile swab into a stool sample that presents the most secretion under visual inspection. Then, the swab was inserted into the sample collection tube containing assay diluents. Swab was swirled for 10 times until the sample has been dissolved and discarded while squeezing against the wall of the tube. A dropping cap was assembled on the sample collection tube and the tube was left for settling for 5 minutes. After, the test device was removed from the foil pouch and placed on a flat and dry surface. Three drops (about 80 µl) of the mixture were added into the sample well(s) of the test device. As the test begins to work, purple color move across the result window in the center of the test device. Test results were interpreted after 10-15 minutes. Results were interpreted as negative if the presence of only control band (C) with the result window were seen. Positive result includes the presence of two color bands as test band (T) and control band (C) within the result window, no matter which band appears first, indicates a positive result. Results were invalid if the control band (C) is not visible within the result window after performing the test this needs to be re-tested using a new test kit.

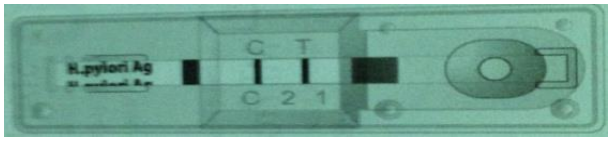

Figure 3. Positive result for *H. pylori* antigen using HpSA

Statistical Analysis

Statistical analysis was carried out using the SPSS statistical software package version 16.0. Data were expressed as mean ± standard deviation (SD) or number and percentage of the subjects. The McNemar Test was used to compare the nominal data obtained from participants' results in HpSA with UBT and determine their significant difference.

Limitations of the study

Sampling was done during the last week of January 2016 up to end of February 2016. Although, the patients were from the gastro intestinal clinic, the underlying clinical conditions of the participants who were included in the study were not assessed. Moreover, the medications taken, if there are, by the participants were not also determined.

RESULTS AND DISCUSSION

Table 1
Summary Table of Results Stratified by Age Using ELISA IgG

Age	(-)	%	B	%	(+)	%
13 – 18 y.o.	7	6.7	2	1.9	6	5.7
19 – 59 y.o	19	18.3	3	2.9	62	59.6
60 & above	2	1.9	1	0.1	2	1.9
Total	28	26.9	6	5.8	70	67.3

Legend: (-) – negative result (<16 RU/mL) (B) – borderline (≥16 - <22 RU/mL) (+) – positive result (≥22 RU/mL)

Table 1 shows the summary of the results in ELISA IgG. As shown, many participants (67.3%) were found to be positive on the test with IgG levels of equal to or above 22 RU/mL, and more than half (59.6%) of those who were found positive for *H. pylori* belong to adults group. This shows that majority of participants with *H. pylori* infection were adults which implies that *H. pylori* infection is really common among such group.

There were also borderline results (5.8%) observed. This confirms the fact that *H. pylori* infection reaches up to 60% in older ages as revealed by Salih [4]. These findings support the results of Marie [24] that *H. pylori* seroprevalence was increasing with age. It is estimated that *H. pylori* infection affects more than half of the adult population worldwide and is responsible for 75% of all gastric cancer cases [25]. The similar phenomenon was found in other studies Bakka & Salih, [26] where asymptomatic subjects >40 years of age have shown 75-85% seropositivity *for H. pylori.* This finding further substantiates the age of acquisition of *H. pylori* since infection is a long-term chronic infection [27].

Table 2 shows the results in HpSA and UBT. Among adolescents (13-18 years old), there was one participant who was found to be positive in UBT but turned to be negative in HpSA.

Table 2
Summary Table of Results Stratified by Age Using HpSA and UBT

Age	HPSA		UBT	
	(-)	(+)	(-)	(+)
13 – 18 y.o.	11 (10.6%)	2 (1.9%)	10 (9.6%)	3 (2.9%)
19 – 59 y.o	58 (55.8%)	28 (26.9%)	57 (54.8%)	29 (27.9%)
60 & above	5 (4.8%)	(0%)	4 (3.8%)	1 (1.0%)
Total	74 (71.2%)	30 (28.8%)	71 (68.2%)	33 (31.8%)

Legend: HPSA: (-) – negative result (+) – positive result
UBT: (-) – negative result (<50 cpm)
(+) – positive result (≥50 cpm)

The result is similar with the adults (19-59 years old) and senior adult participants (60 years old and above). The grouping for age was based on Neutral Network using FG-NET Aging Database. However, most of the obtained results in HpSA were similar with UBT. In general, it is noticeable that most of the results obtained in UBT were similar with that of HpSA. This implies that HpSA has similar sensitivity with UBT in detecting *H. pylori* from the participants' samples. This supports the study of Choi, et al., in 2011, to the performance of a new stool antigen test was comparable to that of other methods such as UBT in the diagnosis of *H. pylori* infection for the screening population.

Table 3
Comparison on the Results of ELISA IgG with HpSA and UBT

	ELISA IgG & HpSA	ELISA IgG & UBT
N	104	104
Chi-square[a]	35.027	36.214
Asymp. Sig.	.000	.000

Table 3 shows the comparison on the results obtained on ELISA IgG with HpSA and UBT in detecting *H. pylori*. Using McNemar test, results revealed a p value of 0.000 which indicate that there is a significant difference between the results obtained in ELISA IgG and HpSA and UBT. This proves that UBT and HpSA are better diagnostic procedures than ELISA in detecting *H. pylori*. With the actual results obtained, all the negative results in ELISA IgG turned out negative when confirmed using HpSA and UBT. However, there were positive results in ELISA IgG that became negative after confirmation using the two tests. This implies that ELISA IgG is senstive in detecting negative results but not in detecting positive results.

In the hospital setting where the study was conducted, ELISA serology for *H. pylori* detection is the method employed in screening the patients with gastrointestinal diseases. According to the test instruction provided [11], the test is less accurate and does not identify active infection. Thus, the method is only used as a screening procedure. It is a reliable predictor of infection in high prevalence developing countries but is not recommended after therapy.

The findings also show that UBT must be used to confirm a positive screening result especially that the clinical conditions of the participants were not determined. According Manes et al. [17], UBT can obviate the need for antral biopsies to confirm eradication of *H. pylori* after completion of treatment [15]. They added that UBT is a very accurate test for detecting *H. pylori* infection with sensitivity and specificity better than many other tests for *H. pylori.* In view of its high sensitivity and specificity, the UBT is useful for rapid, non-invasive diagnosis of *H. pylori* infection [18].

The comparison on the results of HpSA and UBT is presented in Table 4. The percentage of positive sample on UBT is 32.4%, but for HpSA the positive result dropped to 28.8%. The reduction is not statistically significant since the computed p-value of 0.375 is greater than 0.05 alpha level using McNemar test. Therefore, the results obtained in HpSA are comparable with UBT in terms of confirming the results of ELISA IgG.

Table 4
Comparison on the Results of HpSA and UBT

			HpSA (-)	HpSA (+)	Total
UBT	Negative	Count	70	1	71
		% within UBT	98.6%	1.4%	100.0%
		% within HpSA	94.6%	3.2%	67.6%
	Positive	Count	4	29	33
		% within UBT	12.1%	87.8%	100.0%
		% within HpSA	5.4%	96.8%	32.4%
	Total	Count	74	30	104
		% within UBT	71.2%	28.8%	100.0%
		% within HpSA	100.0%	100.0%	100.0%

Stool antigen test is a simple non-invasive and accurate test for the diagnosis of *H. pylori* infection [28] that can possibly represent a viable alternative to the UBT for the primary diagnosis of *H. pylori* infection and for monitoring treatment outcome [29]. *H. pylori* stool test represents an accurate and novel non-invasive concept for diagnosis of infection and can be used for daily routine in clinical practice [30]. She et al. [31] also showed that HpSA can be used as the gold standard as it is offers excellent sensitivity and specificity when compared to invasive methods.

Though UBT remains the first-line non-invasive test in the assessment of the presence of *H. pylori* infection, and that according to the experts who participated in the Maastricht 2-20000 Consensus Conference, HpSA should only be used when UBT is not available [13]. The current findings proved that HpSA can be used as an alternative to UBT in confirming *H. pylori* due to the comparable results obtained.

CONCLUSION AND RECOMMENDATION

The study revealed that many participants were positive for H. pylori. There is a difference between the results obtained in ELISA IgG with that of HpSA and UBT, proving that the two diagnostic procedures are better than ELISA in providing accurate results. There is no significant difference between HpSA and UBT which shows that they are comparable. Thus, HpSA can be used as an alternative confirmatory test in detecting H. pylori in the Kingdom of Saudi Arabia.

Based on the results, the researcher recommends that the stool antigen assay (HpSA) be used as a cheaper alternative confirmatory to the expensive Urea Breath test in detecting H. pylori. Future researchers can utilize the demographic profile of their participants and perform correlation to identify possible factors that can affect H. pylori detection.

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