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COMPARISON STUDY BETWEEN THREE NON INVASIVE METHODS USED FOR DIAGNOSING *HELICOBACTER PYLORI* INFECTION

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ABSTRACT
Background: *Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerophilic bacterium it is one of the most common human-specific pathogens which exclusively inhabits the gastric mucosa. Infection with *H. pylori* is always associated with chronic gastric inflammation, gastritis and peptic ulceration which can lead to gastric cancers such as adenocarcinoma. The aim of this study was to compare between three non invasive methods used for diagnosing *Helicobacter pylori* infection. **Methodology**: this study was conducted in Khartoum state from February to May 2017. Stool and serum samples were collected from 105 patients with gastric disorders. The presence of *H.pylori* infection was evaluated by urea breath test, stool antigen detection test and antibody detection test. **Results:** A total number of 45 patients gave positive result in urea breath test while 60 patients' showed negative result.44 patients gave positive result in stool antigen test while 61 patients showed negative result. A total number of 40 patients gave positive result in antibody detection test while 65 patients' showed negative result. The result showed that the similarity between urea breath test, stool antigen test, urea breath test, antibody detection test and stool antigen test, antibody detection test was 99%, 72,4% and 71,4 % respectively. While the similarity between these three method was 69.5%. **Conclusion:** In this study there was no significant differences between these three non invasive methods. The combination of antibody detection and stool antigen test or using urea breath test only can significantly improve the detection of *H. pylori* infection.

KEYWORDS: *H. pylori*, urea breath test, stool antigen test, antibody detection test.

INTRODUCTION
Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic bacterium which was identified in 1982 by Marshall and Warren.[1,2] *H. pylori* is one of the most common human-specific pathogens which exclusively inhabits the gastric mucosa.[3] Infection with *H. pylori* is always associated with chronic gastric inflammation, gastritis and peptic ulceration which can lead to gastric cancers such as adeno carcinoma, lymphoma of the stomach or benign mucosal-associated lymphoid tissues (MALT).[4,5] *H. pylori* infection is prevalent throughout the world and more than half of the world population harbors this organism.[6] There is a higher incidence of infection in less developed and developing countries.[7,8]

The appearance of symptoms of *H. pylori* infection varies depending on the strains of *H. pylori* and the interaction of both bacterial and host factors. However, most *H. pylori*-infected persons are asymptomatic due to cofactors shortage of the host or bacteria or colonization by less virulent strains.[11,12] The spiral shape, motility and production of urease are important virulence factors of *H. pylori* which facilitate the colonization of bacterium in the stomach mucosa.[11] Furthermore, the bacterium releases several pathogenic proteins such as cytotoxin-associated antigen (Cag A) and vacuolating cytotoxin (Vac A).[13] The cytotoxin-producing strains of *Helicobacter* contains the cag A gene (type I strains) and are frequently isolated from patients with gastric diseases. Hence, the detection of cag A is used for identifying infection with harmful strains.[14] A number of methods are currently available for detection of *H. pylori* infection that divided into two groups of invasive and noninvasive methods. Invasive techniques include biopsy-based histological methods, culture of the biopsy, the rapid urease test and molecular tests (e.g. real-time PCR).[15] Non invasive methods encompass the ^{13}C-urea breath test (^{13}C-UBT), The ^{13}C-UBT is considered the non-invasive gold standard method of H. pylori diagnosis.[16–17] it is a simple and safe test, which is easily repeated and provides excellent accuracy for the initial diagnosis of H. pylori infection, the test is based on this. Patients ingest urea labeled with either ^{13}C or ^{14}C and the labeled urea comes into contact with the mucosa and diffuses through the mucus towards the *H. pylori* and the mucosal blood supply, urea hydrolysis occurring

within the mucous layer produces ammonia and labeled CO_2. The radioactivity of each sample is measured by a scintillation counter and the results are expressed as a percentage of the administered dose, adjusted for endogenous CO_2 production, or directly as counts/min.[18,19] The stool antigen test is considered as a valuable noninvasive alternative to diagnose *H. pylori* when UBT is not available.[20] Because Endoscopy is expensive, unpleasant to patients and carries a small but definite risk, non invasive tests to assess *H.pylori* infection have therefore been used.[17]

The aim of this study was to compare between three non invasive methods used for diagnosing *Helicobacter pylori* infection.

MATERIALS AND METHODS

Patients

This study consisted of 105 adult patients that presented with dyspeptic symptoms to the gastroenterologist in Total Lab care laboratory and Ultra Lab laboratory in Khartoum City. The inclusion criteria of the patients were evaluated by the gastroenterologist. The exclusion criteria of the patients were as follows: previous gastric surgery and/or *H. pylori* eradication treatment, recent use of bismuth-containing compounds or antibiotics (in the last 2 months) or proton pump inhibitors (in the last 4 weeks), long-term use of corticosteroids and/or immunosuppressant, pregnancy and lactation. A history of bleeding and coagulation disorders included in the exclusion criteria. Verbal informed consent was provided from all patients.

Samples

Stool sample was collected to detect antigens and serum sample was collected to detect antibodies.

Urea breath test

The ^{13}C-UBT was performed according to the manufacturer's instructions (HUBT-20). after 2 hours fasting period, breath samples were obtained before (baseline) and 30 min after the test drink intake (75 mg ^{13}C-urea 5from the capsule dissolved in 200 mL fruit juice) and after a period of 15 min each of the subjects were given a heliprobe breath card to deeply blow air through the card from their mouth until there was adequate color change on the breath card. The breath card was then read using heliprobe machine to determine the presence of *H. pylori* infection in the subject.

H. pylori stool antigen (HpSA) test

The patients provided fresh stool samples in air tight containers then stool antigen test was performed. The test was performed according to the manufacturer's instructions (The Bio Tracer™), By using the applicator stick of the provided sample diluents vial, a small portion of stool specimen was transferred into the sample diluents and mixed well by shaking gently. The tip of the vial was broken off, and five drops (150 µL) were added to the sample well in the test device. The test result was readed after 10 minutes. A positive test result was indicated by the appearance of red band in the zone marked C (control line) and a red band in the zone marked T (test line). The sample was considered negative when only one red band appeared across the central window in the zone marked C.

H. pylori antibody detection test

Blood samples were collected in plain container to obtain serum, The test was performed according to the manufacturer's instructions (The EcoTEST™) the test device was placed on clean, flat surface, then the plastic dropper was filled with the specimen, the dropper was holded vertically and 1 drop of serum was dispensed into the sample well, then 1 drop of sample diluents was added with bottle positioned vertically. Result was readed in 15 minutes. The color was migrated across the result area in the center of the device. A positive test result was indicated by the appearance of red band in the zone marked C (control line) and a red band on the zone marked T (test line). The sample was considered negative when only one red band appeared across the central window in the zone marked C.

RESULTS

A total of 105 patients with dyspeptic symptoms were included in the study. Of these patients, 77/105 (73.3%) were male and 26/105 (26.7%) were female. From these patients stool samples were taken for the detection of *H. pylori* antigen, blood for detection of anti- *H. pylori* IgG antibodies and urea breath test was performed. A total number of 45 patients gave positive result in urea breath test while 60 patients' showed negative result (Table 1). 44 patients gave positive result in stool antigen test while 61 patients showed negative result (Table 2). A total number of 40 patients gave positive result in antibody detection test while 65 patients' showed negative result (Table 3). The result showed that the similarity between urea breath test, stool antigen test, urea breath test, antibody detection test and stool antigen test, antibody detection was (99%, 72.4% and 71.4%) respectively (Table 4,5 and 6).While the similarity between these three method was 69.5%. (Table 7).

Table 1. The results of urea breath test.

Result	Frequency	Percent
+ ve	44	41.9
- ve	61	58.1
Total	105	100.0

Table 2. The result of stool antigen detection test (HpAg).

Result	Frequency	Percent
+ ve	45	42.9
- ve	60	57.1
Total	105	100.0

Table 3. The result of antibody detection test (HpAb).

Result	Frequency	Percent
+ ve	40	38.1
- ve	65	61.9
Total	105	100.0

Table 4. UBT-HpAg Level of Matching.

Result	Frequency	Percent
similarity	104	99
difference	1	1
Total	105	100.0

Table 5. UBT-HpAb Level of Matching.

Result	Frequency	Percent
similarity	76	72.4
difference	29	27.6
Total	105	100.0

Table 6. HpAg-HpAb Level of Matching.

Result	Frequency	Percent
similarity	75	71.4
difference	30	28.6
Total	105	100.0

Table 7. UBT-HpAg-HpAb Level of Matching.

Result	Frequency	Percent
similarity	73	69.5
difference	32	30.5
Total	105	100.0

DISSCUSION

Continuous developments in both invasive and non-invasive based methods for detection of *H. pylori* infection will greatly contribute to further improvement of the health management *H. pylori* associated disorders. Since, in the present study we compared between the result that obtained from different non invasive procedures used for detection of *Helicobacter pylori* infection which include the urea breath test, stool antigen detection and antibody detection. The result showed the there was no significant different between the result of urea breath test and stool antigen detection test (similarity was 99% as showed in table 4), and little but not significant difference between the result of urea breath test and antibody detection test (similarity was 72.4% as showed in table 5) and no significance difference between the result of stool antigen detection test and antibody detection test (similarity was 71.4% as showed in table 6) and there was a little but not significant difference between this three test (similarity was 69.5% as showed in table 7), This results were agreed with result that obtained in research submitted by Monteiro L et l in 2001.[21] In a study by Dietmer Enko et al the result showed there was significant difference between results of urea breath test and antibody detection test, in total of 108 samples 32 samples showed positive by urea breath test compared with 3 samples showed positive by antibody detection test and 17 samples showed negative result compared with 56 samples respectively.[22] the sample size. Patients with previous eradication therapy and various populations with different geographical and socio-economic status, may be the possible explanation of discrepant test results between the different studies.

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CONCLUSION

Based on our findings, there was no significant differences between these three non invasive methods. The combination of antibody detection and stool antigen test or using urea breath test only can significantly improve the detection of *H. pylori* infection.

REFERENCES

1. Ahmed N. 23 years of the discovery of *Helicobacter pylori*: Is the debate over? *Ann Clin Microbiol Antimicrob*, 2005; 4: 17. doi: 10.1186/1476-0711-4-17.
2. Ishaq S, Nunn L. *Helicobacter pylori* and Gastric Cancer: A State of the Art Review. *Gastroenterol Hepatol Bed Bench*, 2015; 8(Suppl 1): S6-S14.
3. Logan RP, Walker MM. ABC of the upper gastrointestinal tract: Epidemiology and diagnosis of *Helicobacter pylori* infection. *BMJ*, 2001; 323(7318): 920-2.
4. Thung I, Aramin H, Vavinskaya V, Gupta S, Park JY, Crowe SE, et al. Review article: the global emergence of *Helicobacter pylori* antibiotic resistance. *Aliment Pharmacol Ther*, 2016; 43(4): 514-33. doi: 10.1111/apt.13497.
5. Redeen S, Petersson F, Tornkrantz E, Levander H, Mardh E, Borch K. Reliability of diagnostic tests for *Helicobacter pylori* infection. *Gastroenterol Res Pract*, 2011; 2011: 940650.doi:10.1155/2011/94065.
6. Conteduca V, Sansonno D, Lauletta G, Russi S, Ingravallo G, Dammacco F. *H. pylori* infection and gastric cancer: state of the art (review). *Int J Oncol*, 2013; 42(1): 5-18. doi: 10.3892/ijo.2012.1701.
7. Goddard AF, Logan RPH. Diagnostic methods for *Helicobacter pylori* detection and eradication. *Br J Clin Pharmacol*, 2003; 56(3): 273-83. doi: 10.1046/j.1365-2125.2003.01941.x.
8. van Doorn LJ, Henskens Y, Nouhan N, Verschuuren A, Vreede R, Herbink P, et al. The Efficacy of Laboratory Diagnosis of *Helicobacter pylori* Infections in Gastric Biopsy Specimens Is Related to Bacterial Density and vacA, cagA and iceA Genotypes. *J Clin Microbiol*, 2000; 38(1): 13 7.
9. Falsafi T, Lavasani P, Basardeh I, Massarrat S, Landarani Z. Evaluation of an Iranian Home-made *Helicobacter pylori* Stool Antigen ELISA Kit. *Jundishapur J Microbiol* 2014; 7(6): e10629. doi: 10.5812/jjm.10629.
10. Alborzi A, Soltani J, Pourabbas B, Oboodi B, Haghighat M, Hayati M, et al. Prevalence of

Helicobacter pylori infection in children (south of Iran). *Diagn Microbiol Infect Dis.*, 2006; 54(4): 259-61. doi: 10.1016/j.diagmicrobio.2005.10.012.
11. Dubois A. Spiral bacteria in the human stomach: the gastric helicobacters. *Emerg Infect Dis.*, 1995; 1(3): 79-85. doi: 10.3201/eid0103.950302.
12. Yamaoka Y. Mechanisms of disease: *Helicobacter pylori* virulence factors. *Nat Rev Gastroenterol Hepatol*, 2010; 7(11): 629-41. doi: 10.1038/nrgastro.2010.154.
13. Jones KR, Whitmire JM, Merrell DS. A tale of two toxins: *Helicobacter pylori* CagA and VacA modulate host pathways that impact disease. *Front M*, 2010.
14. Andreson H, Loivukene K, Sillakivi T, Maaroos HI, Ustav M, Peetsalu A, et al. Association of cagA and vacA genotypes of *Helicobacter pylori* with gastric diseases in Estonia. *J Clin Microbiol*, 2002; 40(1): 298-300. doi: 10.1128/JCM.40.1.298-300.2002.
15. Garza Gonzalez E, Perez Perez GI, Maldonado Garza HJ, Bosques Padilla FJ. A review of *Helicobacter pylori* diagnosis, treatment and methods to detect eradication. World J Gastroenterol, 2014; 20(6): 143849. doi: 10.3748/wjg.v20.i6.1438.
16. Sharma TK, Young EL, Miller S, Cutler AF. Evaluation of a rapid, new method for detecting serum IgG antibodies to *Helicobacter pylori*. Clin Chem., 1997; 43(5): 832–6.
17. Parente F, Bianchi Porro G. The (13)C urea breath test for non invasive diagnosis of *Helicobacter pylori* infection: which procedure and which measuring equipment? Eur J Gastroenterol Hepatol. 2001.
18. Savarino V, Vigneri S, Celle G. The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection. Gut. 1999; 45(Suppl 1): I18–22. World J Gastroenterol. 2014; 20(36): 12847–59.
19. Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of *H. pylori* infection: a systematic review and meta-analysis. Am J Gastroenterol, 2006; 101: 1921–30.
20. Issacson PG. gastric lymphoma and *Helicobater pylori*, N Engl J Med, 1994; 330: 1310-1.
21. MonteiroL et al diagnosis of *Helicobacter pylori* infection: non invasive method compared to invasive method and evaluation of two new tests, Am j gastroentrol, 2001 feb; 96(2): 353 -8.
22. Performance evaluation of a rapid whole-blood immunoassay for the detection of IgG antibodies against *Helicobacter pylori* in daily clinical practice 2016 Au 8 Doi: 10.1186/s12941-016-0161-1.