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Original article

Evaluating the Performance of Stool Antigen Tests to Detect *Helicobacter pylori* Infection in Tobruk, Libya

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ABSTRACT

Background and aims. *Infection by Helicobacter pylori (H. pylori) occurs in half of the general population worldwide, with high geographic variability. Even though H. pylori is the leading cause of several gastric diseases, ranging from gastritis and peptic ulcers to gastric malignancies such as gastric cancer and mucosa-associated lymphoid tissue lymphoma, most of the infections remain asymptomatic. Early detection and eradication of H. pylori can definitely prevent severe long-term gastric diseases associated with the bacteria. In Libya, the prevalence of H. pylori is not well documented, especially in healthy subjects. The aim of this study is to evaluate the performance of a stool antigen test for H. pylori in people from Tobruk, Libya.* ***Methods.*** *The present study was conducted on 130 stool samples, including both children and adults. The present study was carried out at the polyclinic in Tobruk, Libya.* ***Results.*** *A total of 130 symptomatic patients selected randomly (50 males and 80 females; age range of 5 to 80 years) were studied. Out of the 130 participants screened, 70 (53.84%) were positive, while 60 (46.15%) were negative for H. pylori stool antigen. To detect H. pylori antigen in feces, the TECAN fecal antigen and the ELISA enzyme-linked immunoassay for the quantitative and qualitative determination of Helicobacter pylori antigen in feces were used.* ***Conclusion****. In this study, the stool antigen test, a low-cost and rapid diagnostic technique, proved to be highly sensitive and specific for detecting H. pylori infection.*

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INTRODUCTION

Helicobacter pylori is a gram-negative, microaerophilic, fastidious bacterium that has infected approximately 50% of humans worldwide over the centuries. Infections frequently occur in childhood and can last a lifetime if not treated. This human pathogen is known to cause a variety of gastric disorders, but it may also be linked to extragastric diseases such as anemia, dyspepsia, and immunological disorders [1].

H. pylori infection is probably the most important factor that has been associated with the development of gastric cancers in human populations. Since its isolation in 1982, evidence for its substantial casual role in the pathogenesis of gastric cancer has substantially increased. *H. pylori* colonizes the stomach and is thought to be the most common chronic bacterial infection in the world [2].

Consequently, *H. pylori* has been identified as a Class I carcinogen and as the most common etiologic agent of infection-associated cancers by the World Health Organization (WHO) and the International Agency of Research on Cancer (IARC) [3]. According to recent global statistics, gastric cancer ranks sixth in incidence and second in mortality among all cancers worldwide [4].

H. pylori is an important cause of chronic active gastritis and plays an important role in the aetiology of peptic ulcer disease in humans. It may be acquired at any age, but once acquired, the infection persists for years and often for a lifetime. The age-specific prevalence of H. pylori infection is higher in developing countries than in developed countries [5].

Several methods have been used to diagnose *H. pylori* infection. Direct methods are dependent on endoscopic gastric biopsy specimens and include rapid urease test (RUT), smear microscopy, culture isolation, histopathology examination, and molecular diagnosis by polymerase chain reaction. The indirect methods include urea breath tests and serology for antigen and antibody detection [6].

This bacterium has developed resistance to stomach acid through colonization in a very narrow place of gastric lactation and secretion of the urease, which breaks down urea located in the medium to ammonia, which has the effect of the acidic acid in the stomach lining, which enables them to stay in the human stomach lifelong if not treated with antibiotics [7].

H. pylori eradication regime includes proton pump inhibitors (PPIs) and antibiotics such as metronidazole, amoxicillin, and clarithromycin. However, antibiotic resistance results in an increased failure rate of therapies [8]. Susceptibility testing of *H. pylori* has become increasingly important for the search for an efficient antimicrobial combination that allows for eradication of this bacterium from the stomach [9].

Diagnosis of *H. pylori* infection can be made by using several invasive or non-invasive techniques. Invasive diagnostic assays include rapid urease testing, histological examination, culture, and polymerase chain reaction (PCR). Non-invasive diagnostic assays include serology tests, urea breath tests, and stool antigens [10].

The aim of this study is to evaluate the performance of a stool antigen test for *H. pylori* in people from Tobruk, Libya, and demonstrate that the HpSA test can replace endoscopy and biopsy for detecting *H. pylori* infection.

METHODS

Study design and setting

The present study was carried out at the polyclinic in Tobruk, Libya. A total of 130 symptomatic patients selected randomly (50 males and 80 females; age range from 5 to 80 years) were studied.

Specimen collection & storage

A fresh fecal sample should be collected into a stool sample collection container. It is required to collect a minimum of a 1-2 mL liquid stool sample or a 1-2 g solid stool sample. The collected fecal sample must be transported to the lab in a frozen condition (-20 ^{o}C). If the stool sample is collected and tested on the same day, it is allowed to be stored at 2-8 ^{o}C.

In the present study, the instrument used was the TECAN Fecal *H. pylori* Antigen ELISA (RE58891) (IBL International GmbH D-22335 Hamburg, Germany) This microplate-based ELISA (enzyme linked immunosorbent assay) kit is intended for the quantitative and qualitative detection of *H. pylori* antigen in feces. The assay is a useful tool in the detection of active *H. pylori* infection. This kit is for in vitro diagnostic use only.

Data collection procedure

All the collected data was entered into Microsoft Excel and cross checked for the presence of any errors to maintain its accuracy. Descriptive statistics were applied to calculate proportions and frequencies. Statistical analysis was performed using IBM SPSS software for Windows version 27 (NY, USA).

RESULTS

The present study investigated the prevalence of *H. pylori* infection among both children and adults in Tobruk, Libya. A total of 130 patients were screened. Out of the 130 participants screened, 68 (52.30%) were positive, while 62 (47.69%) were negative for *H. pylori* stool antigen. The infection rate shown between males and females was (32.5%) and (55.9%), respectively, and there was a significant difference in the prevalence of *H. pylori* with respect to gender shown in (Table 1). The titer over 100 ng/ml was shown in 14 patients, 4 males and 10 females, respectively (18.2%) (Table 2). The prevalence of *H. pylori* infection according to age ranged from (28.57%) in the 5–10-year-olds to (14.28%) in the 11–20-year-olds, (18.57%) in the 21–30-year-olds, (15.71%) in the 31–40-year-olds, and (12.8%) in the 41–50-year-olds, and over 60 years was 10% (Table 3).

Table 1. Gender wise distribution H. pylori of patients.

Gender	Positive	Negative	Percentage
Male	25	25	32.5 %
Female	43	37	55.9 %
Total			88.4 %

Table 2. Results of H. pylori titers greater than 100 ng/ml in patients.

Total.NO	*H. pylori over 100 ng/ml*	Male	Female	Percentage
130	14	4	10	18.2 %

Table 3. Age distribution of H. pylori positive patients.

Age group	No. of patients (70)	Percentage
5-10 years	20	28.57 %
11-20 years	10	14.28 %
21-30 years	13	18.57 %
31-40 years	11	15.71 %
41-50 years	9	12.8 %
>50 years	7	10 %

DISCUSSION

Methods for the detection of *H. pylori* infection are classically divided into invasive and noninvasive. No single test is a fully reliable method for the detection of *H. pylori* in all instances. even though histology is not positive in 100% of infected children [11].

Invasive and non-invasive diagnostic methods for *H. pylori* infection exist. Culture, immunohistochemistry, rapid urease tests, and polymerase chain reaction are invasive methods that require upper gastrointestinal endoscopy to obtain the diagnostic sample. Non-invasive detection methods, on the other hand, include the urea breath test, as well as serological and stool antigen methods. However, invasive tests are thought to be the most accurate of all available tests. Serological methods are used for initial screening and are based on the detection of *H. pylori* specific antibodies in serum, saliva, or urine. When the urea breath test is negative, the stool antigen test is especially helpful [12].

In the present study, the HpSA test had a sensitivity and specificity of 95%, for *H. pylori* screening in children and adults with abdominal pain. Most previous studies in adults and children have compared the efficacy and accuracy of the polyclonal HpSA with the various invasive and non-invasive tests used for diagnosing *H. pylori* infection [13]. We demonstrated high sensitivity, specificity, and likelihood ratios for the polyclonal stool antigen compared with invasive tests in the diagnosis of *H. pylori* infection in children. The sensitivity and specificity of the stool test are reported to be over 90% in children with gastrointestinal symptoms [14].

Study in Libya with general population: 360 asymptomatic individuals aged 1-over 70 years have been investigated and an overall prevalence rate of 76% has been detected in this population, which was age dependent with a 50% infection rate in subjects 1-9 years of age that increased to 84% in subjects 10-19 years and continued with increasing age and reached up to 94% in those over 70 years of age [15]. In symptomatic patients: One hundred thirty-two patients with the symptoms of dyspepsia aged 15-83 years attending the endoscopy unit at the El-Jamahiriya Hospital, Benghazi, Libya, were examined. *H. pylori* was detected in 82% patients. The endoscopic findings revealed that 77% of patients with non-ulcer dyspepsia, and 100% of those with gastric ulcer and in one patient with gastric cancer were *H. pylori* positive [16,17].

Although *H. pylori* infection is the main risk factor for developing gastric cancer and gastric or duodenal ulcers, success in eradication therapy may lead to prevention of these diseases [18].

[19] In addition, eradication therapy for *H. pylori* infection is important for prevention of the spread of infection and may contribute to a reduction in medical expenditure on gastric diseases in the future. *H. pylori* eradication therapy improves gastric mucosal inflammation and atrophy, prevents the progression of intestinal metaplasia, and lowers the incidence of gastric cancer [20,21].

A stool antigen test, which detects present but not previous infection of *H. pylori*, would be applicable in a mass survey. The usefulness of stool antigen tests for the screening of gastric cancer should be examined. However, there is a need for further studies with a greater number of patients for an evaluation of its accuracy in children and adults in developing countries [22].

CONCLUSION

In this study, the stool antigen test, a low-cost and rapid diagnostic technique, proved to be highly sensitive and specific for detecting *H. pylori* infection. Our results are comparable to those reported elsewhere in children and adults and demonstrate that the HpSA test can replace endoscopy and biopsy for detecting *H. pylori* infection.

Conflict of Interest

There are no conflicts of interest.

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تقييم أداء اختبارات مستضد البراز للكشف عن عدوى الملوية البوابية في طبرق، ليبيا

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المستخلص

الخلفية والأهداف. تحدث العدوى بالبكتيريا الملوية البوابية (H. pylori) في نصف إجمالي السكان في جميع أنحاء العالم، مع تباين جغرافي كبير. على الرغم من أن الملوية البوابية هي السبب الرئيسي للعديد من أمراض المعدة، بدءًا من التهاب المعدة والقرحة الهضمية إلى الأورام الخبيثة في المعدة مثل سرطان المعدة وسرطان الغدد الليمفاوية للأنسجة اللمفاوية المرتبطة بالغشاء المخاطي، فإن معظم الإصابات تظل بدون أعراض. من المؤكد أن الاكتشاف المبكر والقضاء على بكتيريا الملوية البوابية يمكن أن يمنع أمراض المعدة الشديدة طويلة المدى المرتبطة بالبكتيريا. في ليبيا، لم يتم توثيق انتشار الملوية البوابية بشكل جيد، خاصة في الأشخاص الأصحاء. الهدف من هذه الدراسة هو تقييم أداء اختبار مستضد البراز لبكتيريا الملوية البوابية لدى أشخاص من طبرق، ليبيا. **طرق الدراسة**. أجريت هذه الدراسة على 130 عينة براز، بما في ذلك الأطفال والبالغين. أجريت هذه الدراسة في العيادة الشاملة في طبرق، ليبيا. **النتائج.** تمت دراسة ما مجموعه 130 مريضاً تظهر عليهم الأعراض تم اختيارهم عشوائياً (50 ذكراً و80 أنثى؛ الفئة العمرية من 5 إلى 80 عاماً). من بين 130 مشاركًا تم فحصهم، كان 70 (53.84٪) إيجابيًا، بينما كان 60 (46.15٪) سلبيًا لمستضد البراز الملوية البوابية. للكشف عن مستضد H. pylori في البراز، تم استخدام مستضد البراز TECAN والمقايسة المناعية المرتبطة بالإنزيم ELISAللتحديد الكمي والنوعي لمستضد Helicobacter pylori في البراز. **الخاتمة**. في هذه الدراسة، أثبت اختبار مستضد البراز، وهو أسلوب تشخيصي سريع ومنخفض التكلفة، أنه حساس للغاية ومحدد للكشف عن عدوى الملوية البوابية.

الكلمات الدالة. هيليكوباكتر بيلوري، اختبار مستضد البراز، إليسا.