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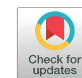
Research article

Evaluation of the diagnostic performance and the utility of *Helicobacter pylori* stool antigen lateral immunochromatography assay



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ABSTRACT

Background: *Helicobacter pylori* causes the most common human gastric infection. *H. pylori* Stool Antigen Lateral Flow Immunochromatography assay (HpSA-LFIA) is considered one of the most cost-effective and rapid non-invasive assays (active tests). Evaluating HpSA-LFIA is of crucial for ensuring accuracy and utility assurance. This study aimed to evaluate the polyclonal antibody-based HpSA-LFIA in comparison to a monoclonal antibody-based ELISA kit.

Methodology: Stool samples were collected from 200 gastric patients for HpSA-LFIA and semiquantitative HpSA-ELISA tests. A statistical analysis of the diagnostic performance was performed using MedCalc software. Chi-square tests were performed to determine the effects of gender and age.

Results and conclusion: The results showed that HpSA-LFIA achieved remarkable sensitivity (93.75%) and NPV (98.00%). However, it had poor specificity, PPV, and accuracy of 59.76%, 31.25%, and 65.31%, respectively. LR+ and LR-were 2.33% & 0.1%, respectively. Gender didn't affect the diagnostic performance of HpSA-LFIA. Age groups had irrelevant sensitivity; however, specificity was significantly higher in patients aged >45 years. We can conclude that HpSA-LFIA was not accurate enough to be the sole test for diagnosis and suggest developing other confirmatory tests in case of positive conditions.

1. Introduction

Helicobacter pylori causes infection characterized by chronic gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma, and extra gastric disorders such as atherosclerosis and skin lesions [1]. *H. pylori* infection ranks among the most common public health problems, affecting approximately 50% of the global population [2].

Identifying *H. pylori* infection is crucial for an appropriate selection of the disease therapy and eradication follow-up protocols. Invasive and non-invasive assays could diagnose the infection. Gastric biopsies are performed in the invasive procedure (endoscopy) to detect *H. pylori* using a rapid urease test, histopathology, Polymerase Chain Reaction (PCR), and culture. The presence of active *H. pylori* infection could be detected via urea breath test (UBT) and stool antigen tests. Serological tests have detected anti-*H. pylori* antibodies, indicating a passive *H. pylori* infection in patients [3].

The invasive approaches for diagnosis are costly, time-consuming. They generally require more than one confirmatory test. On the other hand, the non-invasive approaches could detect both *H. pylori* active and passive infections [2]. Many studies reported that the stool antigen assays are highly sensitive and specific [4]. The European *Helicobacter pylori* study group has recommended the stool antigen test as a non-invasive test for diagnosis [5]. Non-invasive methods as *H. pylori* stool antigen-lateral flow immunochromatography assay (HpSA-LFIA) [6, 7, 8] and enzyme immunoassays (EIA) -as semiquantitative Enzyme-Linked Immunosorbent Assay (ELISA)- are used for stool antigen detection [9, 10]. HpSA-LFIA, a point of care, is preferred due to its fastness, applicability, reliability, and long shelf life at room temperature (12–24 months) [11]. A comparison of commonly used HpSA-ELISA and HpSA-LFIA revealed that HpSA-LFIA had reasonable specificity and sensitivity in children [8].

The meta-analysis studies suggested the superiority of monoclonal antibody-based stool antigen tests compared to polyclonal antibody-based ones in the initial diagnosis of *H. pylori* infection. According to the European Guidelines, monoclonal antibody-based tests and UBT are the most recommended non-invasive assays for monitoring the success or failure of eradication treatment [3, 12]. Although an accurate

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non-invasive test, UBT is comparatively costly and depends upon mass spectrometric analysis, which is not convenient for small centers with limited resources in a few developing countries [13]. Moreover, certain studies accounted for the lower specificity of the UBT in young ages. False-positive results may be attributed to urease-producing bacteria from the oral cavity in non-infected children [14].

HpSA-LFIA could be used as an alternative to UBT to diagnose primary infection of *H. pylori*, especially in developing countries. LFIA is faster than the conventional ELISA, which takes ≥2 h to be performed [8, 15, 16, 17, 18].

Several HpSA-LFIA strips are currently commercially available for the diagnosis of *H. pylori* infection. It is a qualitative test used to detect either anti-*H. pylori* antibodies or *H. pylori* antigens in clinical samples. Both are intended to aid in diagnosing infection in adult patients and following up the infection eradication [2].

This study aimed to evaluate the diagnostic performance of polyclonal-based HpSA-LFIA, the most commercially available assay in Egypt. The evaluation was established using a reference test, monoclonal-based, and semiquantitative double sandwich HpSA-ELISA tests. Statistical analyses were performed to determine the sensitivity, specificity, accuracy, PPV, NPV, LR+, and LR-in different genders and ages.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Research Ethics Committee, Cairo University (approval number, HAM00116).

2.2. Samples

Random stool samples were properly collected from 200 gastric patients (M 80, F 120) from the end of 2019 to summer 2020. The participants age ranged from 3-55 years. The collected samples were divided into three age groups: 13 patients aged 0–19 years, 136 patients aged 20–45 years, and 47 patients aged >45 years, the age distribution is illustrated in Figure 1. According to their physician recommendations, these patients were referred to the clinical laboratories for rapid stool antigen detection with written and assigned consent.

The sample size was calculated with a power of 80%, 95% confidence interval, margin of error 5%, and population proportion 50% (https://www.calculator.net/sample-size-calculator.html). The samples were collected from three Egyptian governorates of Menofia, Benha, and Giza. None of the patients had taken any antibiotics, antacids or proton pump inhibitors (PPIs) one month prior to the sample collection. The stool samples were tested immediately after collection for LFIA and preserved at -20 °C temperature until the ELISA test performance. The samples were transferred to the Microbiology department, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, where the HpSA-ELISA was performed (during the same week of collection).

2.3. H. pylori stool antigen-lateral flow immunochromatography assay (HpSA-LFIA)

Rightsign® *H. pylori* stool antigen rapid test (Hangzhou Biotest Biotech Co., Ltd, Hangzhou, China. Cat.No. R0192c) was applied to the samples according to its pamphlet instructions. The product features were 96.7% sensitivity, 93.8% specificity (https://ctkbiotech.com/product/h-pylori-ag-rapid-test-ce/). This assay used polyclonal anti-*H. pylori* antibodies. The procedures were performed in the following three steps:

1. Sample preparation by stabbing 50 mg of the stool sample from three different sites or 80 μl in diarrheal samples: the samples were then transferred into an extraction buffer.
2. A few drops (80 μl) of the extracted sample were transferred into the LFIA cassette.

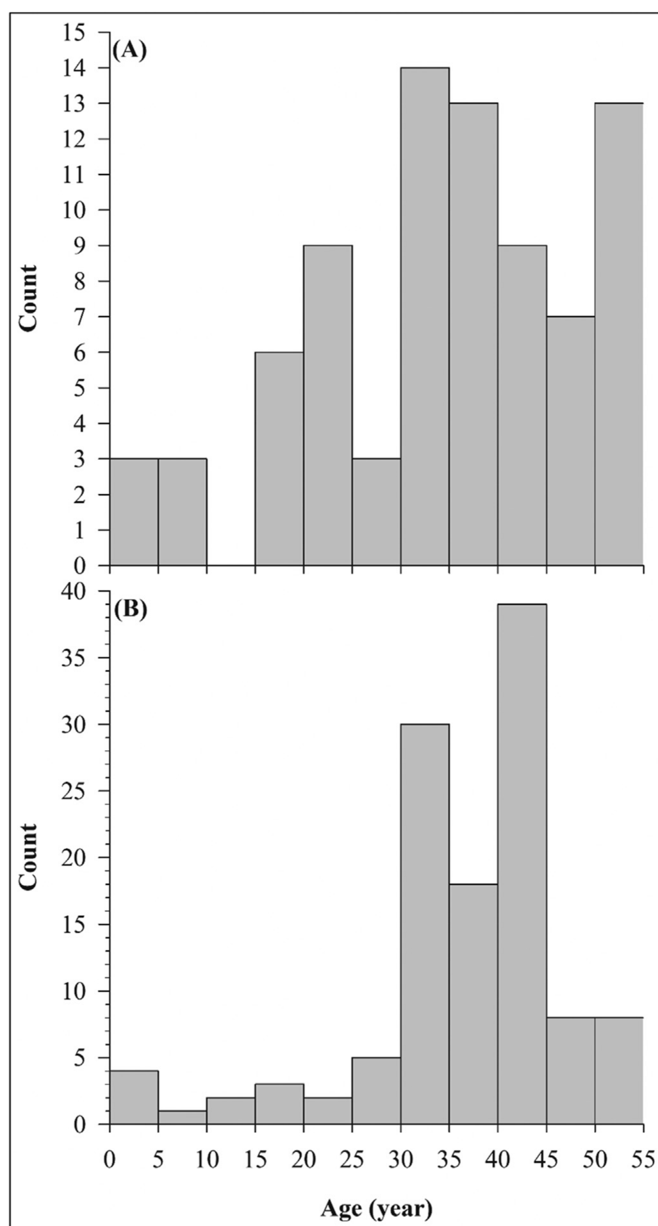

Figure 1. Age distribution (years) of 200 clinically affected patients from whom stool samples were examined a) male (n = 80), b) female (n = 120).

3. The results were read after 10 min of incubation at room temperature.

The results were read by different specialists to avoid an individual-based error.

2.4. H. pylori stool antigen-enzyme immunoassay (HpSA-ELISA)

The *H. pylori* Stool Antigen ELISA kit was used in the study for each sample (FORESIGHT® *H. pylori* antigen EIA test kit, Acon laboratories Inc., 10125 Mesa Rim Road, San Diego, CA 92121, USA, REF 1231-1231). Performance characteristics were 98.6% sensitivity (95% CI: 92.4–100.0%), 95.4% specificity (95% CI: 90.3–98.3%) and overall agreement 96.5% (95% CI: 93.0–98.6%). The procedures were applied according to the manufacturer's protocols. It is a semiquantitative assay, containing *H. pylori* Antigen standard set (0, 5, 10, 25, 50, and 100 ng/ml). In the HpSA-ELISA, a double sandwich assay, the microplates were coated with monoclonal anti-*H. pylori* antibodies. The plate optical densities (ODs) were detected by ELISA reader at 450 nm wavelength.

The optical densities of each sample were determined. The results were obtained by calculating the mean absorbance value of reference standards, specimens, controls, and patient samples. A standard curve was constructed by plotting the mean absorbance obtained from each reference standard (Y-axis) against its concentration in ng/ml (X-axis) (Figure 2). The absorbance values were used to determine the corresponding concentration of *H. pylori* antigen (in ng/ml). Sample concentrations ≥100 ng/ml were considered out of the range of the standard curve (borderline). The test was considered positive if the antigen concentration exceeded 20 ng/ml and negative if the concentration ≤15 ng/ml. The readings between 15-20 ng/ml were considered suspicious, and the sample was retested.

2.5. Statistical analysis

The ELISA test results were used to define *H. pylori* status. Borderline results of four patients were then excluded. For all patients and each sex-age group, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), accuracy, disease prevalence, and their confidence intervals (95% CI) were determined against the defined *H. pylori* status using MedCalc version 20.008 software (www.medcalc.org/calc/diagnostic_test.php). The *Chi*-square test was used to analyze the effects of age and gender on the test performance of HpSA-LFIA. All *p*-values ≤ 0.05 were considered statistically significant. Data are presented as mean ± standard error of the mean (*SE*). Figures were plotted, and statistical analyses were performed using SigmaPlot v14.0 (Systat Software, San Jose, CA, USA).

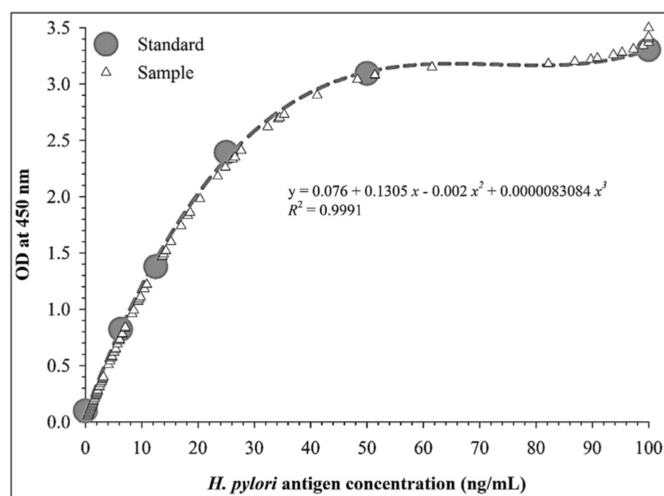

Figure 2. Calibration curve for determination of *Helicobacter pylori* (*H. pylori*) antigen concentration (ng/mL) in stool samples collected from 200 clinically affected patients from their optical density (OD) measured by enzyme-linked immunosorbent assay (ELISA) reader at 450 nm.

3. Results

Lateral flow immunechromatograpgy assay (HpSA-LFIA) of 196 stool samples revealed 100 negative and 96 positive results (four samples were borderline). The readings of the enzyme linked immunesorbant assay (ELISA) kit reported 32 positive results (16.33%), the detailed data of the reading and the results of both ELISA and HpSA-LFIA are demonstrated in ELISA-LFIA file in supplementary data section.

The results comparison between HpSA-LFIA and ELISA showed that 30 samples were true positive (TP). Sixty-six samples were positive LFIA but negative for ELISA (False positive) (FP). Only two samples were negative LFIA, but positive ELISA (False negative) (FN) and 98 samples were negative for both (True negative) (TN). The distribution of TP, FP, FN, and TN among different genders and ages is reported in Table 1. The disease prevalence rate and the statistical diagnostic performance of the HpSA-LFIA in comparison with the HpSA-ELISA (the reference test in this study) are depicted in Tables 2 and 3.

The boxplots of age distributions of males and females tested positive or negative by HpSA-LFIA are illustrated in Figure 3. These boxplots showed the age mean and median of the tested males and females. The age of patients who tested negative ranged between 3 and 55 years (Mean, 37.19 ± 1.61 years; mean ± SE) for males and ranged from 3-55

Table 2. The diagnostic performance of LFIA in comparison to ELISA showing the parameters in all patients and separate gender.

	All	Male	Female
N (%[1])	196 (100.0)	77 (39.29)	119 (60.71)
Sensitivity, % (95% CI)	93.75 (79.19–99.23)	94.44 (72.71–99.86)	92.86 (66.13–99.82)
Specificity, % (95% CI)	59.76 (51.83–67.33)	49.15 (35.89–62.5)	65.71 (55.81–74.7)
PPV, % (95% CI)	31.25 (26.99–35.86)	36.17 (30.1–42.72)	26.53 (21.07–32.82)
NPV, % (95% CI)	98.00 (92.72–99.47)	96.67 (80.92–99.5)	98.57 (91.22–99.78)
Accuracy, % (95% CI)	65.31 (58.19–71.95)	59.74 (47.94–70.77)	68.91 (59.77–77.07)
Disease prevalence, % (95% CI)	16.33 (11.44–22.26)	23.38 (14.48–34.41)	11.76 (6.58–18.95)
LR+ (95% CI)	2.33 (1.89–2.86)	1.86 (1.41–2.44)	2.71 (2.00–3.66)
LR-(95% CI)	0.10 (0.03–0.40)	0.11 (0.02–0.77)	0.11 (0.02–0.72)
Total positive of the reference method, n (%[2])	32 (16.33)	18 (23.38)	14 (11.76)
Total positive, n (%[2])	96 (48.98)	47 (61.04)	49 (41.18)
False negative, n (%[2])	2 (1.02)	1 (1.30)	1 (0.84)
False positive, n (%[2])	66 (33.67)	30 (38.96)	36 (30.25)

[1] Calculated as % of 196 individuals.
[2] Calculated as % of column total (N). 95% CI, 95% confidence intervals; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio for positive test result. LR-, likelihood ratio for negative test result. Borderline results were excluded.

Table 1. The results of LFIA and ELISA of 196 stool samples (4 samples are borderline) and the distribution among different sex and age groups.

		LFIA												Total
		Positive						Negative						
		Male			Female			Male			Female			
		0-19y	20-45y	>45y	0-19y	20-45y	>45y	0-19y	20-45y	>45y	0-19y	20-45y	>45y	
ELISA	Positive	3	11	3	2	8	3	1	0	0	0	1	0	32
	Negative	2	20	8	3	29	4	0	18	11	2	49	18	164
	Total	5	31	11	5	37	7	1	18	11	2	50	18	196

Table 3. The diagnostic performance of LFIA in comparison to ELISA showing the statistical parameters in three age groups among all patients and separate gender.

	All			Male			Female		
	0–19 years	20–45 years	>45 years	0–19 years	20–45 years	>45 years	0–19 years	20–45 years	>45 years
N (%[1])	13 (6.63)	136 (69.39)	47 (23.98)	6 (3.06)	49 (25.00)	22 (11.22)	7 (3.57)	87 (44.39)	25 (12.76)
Sensitivity, % (95% CI)	83.33 (35.88–99.58)	95.00 (75.13–99.87)	100.0 (54.07–100.0)	75.00 (19.41–99.37)	100.0 (71.51–100.0)	100.0 (29.24–100.0)	100.0 (15.81–100.0)	95.00 (51.75–99.72)	95.00 (29.24–100)
Specificity, % (95% CI)	28.57 (3.67–70.96)	57.76 (48.24–66.87)	70.73 (54.46–83.87)	0.00 (0.00–84.19)	47.37 (30.98–64.18)	57.89 (33.50–79.75)	40.00 (5.270–85.34)	62.82 (51.13–73.50)	81.82 (59.72–94.81)
PPV, % (95% CI)	50.00 (35.67–64.33)	27.94 (23.46–32.92)	33.33 (23.7–44.59)	60.00 (46.00–72.54)	35.48 (28.92–42.65)	27.27 (18.12–38.85)	40.00 (24.58–57.69)	21.62 (16.01–28.53)	42.86 (23.61–64.54)
NPV, % (95% CI)	66.67 (19.07–94.44)	98.53 (90.79–99.78)	100.0	0.00	100.0	100.0	100.0	98.00 (88.45–99.68)	100.0
Accuracy, % (95% CI)	53.85 (25.13–80.78)	63.24 (54.55–71.33)	74.47 (59.65–86.06)	50.00 (11.81–88.19)	59.18 (44.21–73.00)	63.64 (40.66–82.80)	57.14 (18.41–90.10)	65.52 (54.56–75.39)	84.00 (63.92–95.46)
Disease prevalence, % (95% CI)	46.15 (19.22–74.87)	14.71 (9.22–21.79)	12.77 (4.83–25.74)	66.67 (22.28–95.67)	22.45 (11.77–36.62)	13.64 (2.91–34.91)	28.57 (3.67–70.96)	10.34 (4.84–18.73)	12.00 (2.55–31.22)
LR+ (95% CI)	1.17 (0.65–2.10)	2.25 (1.78–2.85)	3.42 (2.12–5.50)	0.75 (0.43–1.32)	1.90 (1.41–2.57)	2.38 (1.4–4.02)	1.67 (0.81–3.41)	2.39 (1.65–3.46)	5.50 (2.27–13.35)
LR-(95% CI)	0.58 (0.07–4.95)	0.09 (0.01–0.59)	0.00	–	0.00	0.00	0.00	0.18 (0.03–1.13)	0.00
Total positive of the reference method, n (%[2])	6 (46.15)	20 (14.71)	6 (12.77)	4 (66.67)	11 (22.45)	3 (13.64)	2 (28.57)	9 (10.34)	3 (12.00)
Total positive, n (%[2])	10 (76.92)	68 (50.00)	18 (38.30)	5 (83.33)	31 (63.27)	11 (50.00)	5 (71.43)	37 (42.53)	7 (28.00)
False negative, n (%[2])	1 (7.69)	1 (0.74)	0 (0.00)	1 (16.67)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.15)	0 (0.00)
False positive, n (%[2])	5 (38.46)	49 (36.03)	12 (25.53)	2 (33.33)	20 (40.82)	8 (36.36)	3 (42.86)	29 (33.33)	4 (16.00)

[1] Calculated as % of 196 individuals.
[2] Calculated as % of column total (N). 95% CI, 95% confidence intervals; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio for positive test result. LR-, likelihood ratio for negative test result. Borderline results were excluded.

years (Mean, 37.63 ± 0.99 years) for females. While the age of patients who tested positive ranged from 3.5-45 (Mean, 28.69 ± 3.49 years) for males and ranged from 4-54 (33.14 ± 4.00 years) for females. The probability (*P-value*) of gender effect on the test's sensitivity and specificity was 0.59 and 0.29, respectively. Further, the *P-values* of the age effect on sensitivity and specificity were 0.19 and 0.02, respectively.

4. Discussion

This study evaluates and reports the diagnostic performance (Se, Sp, PPV, NPV, LR+, LR-, and accuracy) of Egypt's most common non-invasive test. Rightsign® *Helicobacter pylori* stool Antigen rapid test was compared to Foresight® semiquantitative HpSA-ELISA. The latter is considered a specific and sensitive test as it could detect low antigen concentrations (0.5 ng/ml).

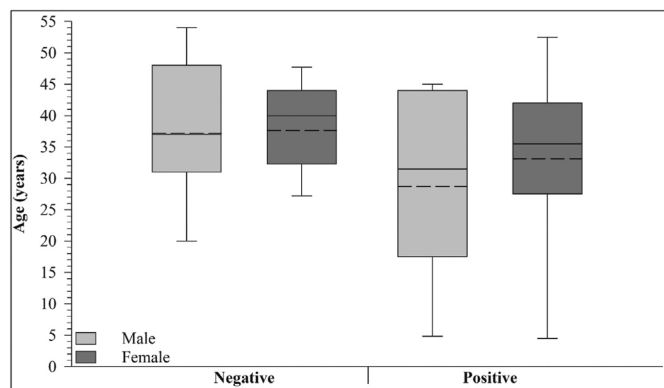

Figure 3. Boxplots of age distributions of males and females tested positive or negative by lateral flow immunoassay tests. Dashed lines indicate mean while solid lines indicate median. Four individuals were removed as they were borderline (3 males; 1 female).

The results of HpSA-LFIA showed poor specificity (Average, 59.76%) but revealed a good sensitivity (Average, 93.75%). These findings did not match the product features of the Rightsign *H. pylori* Ag rapid test, as our results reported a dramatic drop in test specificity. The precision of the HpSA-LFIA indicated un-satisfactory PPV (Average, 31.25%). Nevertheless, it had an acceptable NPV (Average, 98.00%) level. The accuracy of the HpSA-LFIA (Average, 65.31%) was not promising to confirm the diagnosis but sufficient to exclude the disease (Table 2).

The likelihood ratio (LR) assesses the utility of the LFIA and how likely the patient is infected. HpSA-LFIA had low LR+ (2.33), which indicated a low possibility of true positive cases. Contrarily, it had a reliable LR- (0.10), which implied a low possibility of false-negative cases.

The results of HpSA-LFIA reported no statistical significance of gender in sensitivity ($t4 = 0.59$, $P = 0.5882$) or specificity ($t4 = 1.23$, $P = 0.2861$).

The highest HpSA-LFIA diagnostic performance were obtained in elders aged >45 years old. In young aged <18 years, PPV, false positive, and false negative demonstrated the highest values. The specificity in young was modest (Table 3), as documented by **Frenck *et al.*,** who reported significantly lower specificity among Egyptian children ≤6 years [19]. Age groups had no significant effect on sensitivity ($F2.6 = 2.21$, $p = 0.1911$). However, specificity was significantly higher in elders (≥45 years) than younger (≤18 years) ($F2.6 = 8.42$, $p = 0.0183$).

The previous results agree with **da Silva, Kato *et al.*,** and others who recorded that HpSA-LFIA presented 52.5–94.6% sensitivity, 55.5–98.4% specificity, and 98.4% NPV; however, disagree in PPV value (94.6%) [1, 2, 8, 16, 17, 18]. Other similar studies used an insufficient sample size

(65 stool samples) with the ImmunoCard STAT! HpSA assay, obtained 77.8% sensitivity, 79.3% specificity, 82.4% PPV, and 74.2% NPV [20].

Our findings are not in accord by **Karakus, Salih, and Kato *et al.*,** who reported that the HpSA-LFIA was valid for *H. pylori* infection diagnosis in children and adolescents, with comparable results to ELISA. They suggested high accuracy of HpSA-LFIA for all age groups with Se% of 93% and Sp% of 91%. They reported that in a 5-years follow-up study performed in adults, the HpSA-LFIA showed a sensitivity of 93% and a specificity of 100% [8]. **Karakus and Salih** revealed that the sensitivity was 90–100% (average 95%), and the specificity was 80–100% (average 96%) [2]. Another study on 91 patients showed that the sensitivity of the *H. pylori* stool antigen test was 73.9%, and the specificity was 86.7% [21]. Our results also did not trust the assessment of the pre-and post--eradication diagnostic performance of HpSA-LFIA compared to HpSA ELISA in children, which found that sensitivity, specificity, PPV, and NPV for the HpSA-LFIA were 94.6%, 98.4%, 94.6%, and 98.4%, respectively [18]. Evaluation of the diagnostic accuracy of HpSA-LFIA (LİNEAR Chemical, Barcelona, Spain) in 109 children with abdominal symptoms (age range, 5–17; mean, 12.1 years) obtained 65.00% sensitivity and 92.3% specificity [22]. An evaluation of HpSA-LFIA in symptomatic children revealed a sensitivity of 88.9% (95% CI = 77.3–96.3%) and a specificity of 94.0% (95% CI = 88.1–97.7%). There was no age-dependency of the stool test results [7, 23].

Geographical differences perhaps affect the results of HpSA-LFIA and may be able to comprehended the enormous varity in the diagnostic performance, as it was an imported kit. This finding was supported by **Makristathis *et al.*,** who reported that HpSA-LFIA prepared with polyclonal anti-*H. pylori* antibodies varied in sensitivity results, 96.6% in Brazilian children, 91.5% in Italian children, and 67% in Italian adults. However, the Se% in Egyptian adults was 57.7% [9]. *H. pylori* mediates natural transformation and mechanisms of bacterial DNA horizontal gene transfer, which maintain a high level of genetic variability [24]. *H. pylori* has a higher mutation rate than most bacteria [25].

Twenty-two studies (including 2,499 patients) evaluated the monoclonal stool antigen tests before eradication therapy. Pooled sensitivity, specificity, LR+, and LR− were: 94% (95% CI = 93–95%), 97% (95% CI = 96–98%), 24 (95% CI = 15–41), and 0.07 (95% CI = 0.04–0.12), respectively. The accuracy of both monoclonal and polyclonal stool antigen tests was examined together in 13 pretreatment studies, and higher pooled sensitivity was demonstrated with the monoclonal technique (95% vs. 83%). Twelve studies (including 957 patients) assessed the monoclonal stool antigen tests to confirm eradication after therapy. Pooled Sensitivity, Specificity, LR+, and LR− were 93% (95% CI = 89–96%), 96% (95% CI = 94–97%), 17 (95% CI = 12–23), and 0.1 (95% CI = 0.07–0.15), respectively. Both tests were evaluated together in 8 post-treatment studies, and once more, the monoclonal technique showed a higher sensitivity (91% vs. 76%) [12]. Numerous studies have confirmed obtaining better results for invasive vs. non-invasive tests. For a more accurate diagnosis, we suggest not to solely rely on non-invasive methods of *H. pylori* diagnosis [21].

The strength points of our study were using a suitable and calculated sample size of diseased patients. Importantly, the sample size was more than any study in prior literature [8, 15, 16, 17, 19, 20, 21]. An accurate test (ELISA) was used as a reference test to evaluate HpSA-LFIA. Moreover, the study was conducted across age groups and genders. Various statistical parameters were determined to measure the diagnostic performance and utility. However, more comparisons with other invasive and non-invasive tests and larger sample sizes from more Egyptian governorates are highly recommended. These points will be considered in our future studies.

5. Conclusion

HpSA-LFIA in Egypt is a highly sensitive test with low specificity and low accuracy to be the sole test for diagnosis. The test was intense to be used as a screening test and provides a preliminary result that is inadequate for precision and final diagnosis. There is an urgent demand for developing an accurate, rapid monoclonal antibody-based LFIA from local *H. pylori* isolates.

Declarations

Author contribution statement

Shaymaa Abdelmalek: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hisham Abdelrahman: Analyzed and interpreted the data; Wrote the paper.

Wafy Hamed: Conceived and designed the experiments; Performed the experiments.

Neven Nagy: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Karim Shokry: Performed the experiments; Wrote the paper. </p>

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e09189.

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