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Non-invasive follow-up of Egyptian patients infected with *Helicobacter pylori* by quantification of *H. pylori* circulating antigen in serum using ELISA

Running title: Circulating antigen for follow-up of *H. pylori* infection

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Abstract

Clinicians still wish to determine if *H. pylori*-infected patients have been cured after specific treatment. The present study aimed to evaluate the reliability of the *H. pylori* circulating antigen (HpCAg) test for noninvasive screening of *H. pylori* infection and assessment of cure after specific treatment. Sera of 134 symptomatic individuals (81 males & 53 females, aged 23-68 yr) were screened for HpCAg using ELISA. *H. pylori* infection was confirmed using a gold standard based on culture, rapid urease test, and histology testing. The detection rate of HpCAg was 69% among screened individuals. The gold standard confirmed *H. pylori* infection in 93% of individuals showing HpCAg in their sera. In addition, 31% of infected patients were excluded for their drug resistance. Eligible individuals received a standard triple therapy regimen including Lansoprazole, Clarithromycin, and Amoxicillin twice daily for 14 days. Six weeks later, the HpCAg testing was repeated to evaluate the treatment outcome. HpCAg was not detected in 78 % of treated individuals. Furthermore, the levels of HpCAg were significantly decreased ($p < 0.001$) in the sera of non-responders. In conclusion, the detection of HpCAg is a reliable non-invasive approach for screening and follow-up of *H. pylori*-infected individuals after treatment, especially in developing countries.

Keywords: *H. pylori*, Serum, Circulating Antigen, Follow-up, ELISA.

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1. Introduction

Chronic infection with *Helicobacter pylori* (*H. pylori*) is a major cause of gastritis and gastric ulcer disease in humans **(McColl, 2010; Eusebi et al., 2014)** and is a risk factor for the development of gastric cancer **(Cubiella et al., 2021).** Recent studies about *H. pylori* infection worldwide have shown that bacterium spreading is still ongoing **(Crowe, 2019).** More than 50% of people worldwide are thought to carry *H. pylori* in their upper gastrointestinal tracts **(Hooi et al., 2017).** There is a dearth of published data on the prevalence of *H. pylori* in Egypt, which is thought to be between 70% and 80% **(Alboraie et al., 2019).** It is recommended that all patients with documented *H. pylori* infection should be treated with appropriate antibacterial treatment **(Georgopoulos et al., 2013, O'Connor et al., 2015)**. Due to the emergence of antibiotic-resistant strains during the past ten years, it has been demonstrated that antimicrobial therapy is not always totally successful against *H. pylori* infection. **(Ierardi et al., 2013).** Hence, clinicians may wish to determine if the patients have been cured of *H. pylori* after treatment **(Gisbert JP, 2020).** To accurately diagnose *H. pylori* infection, gastric biopsy specimens should be examined via an upper gastrointestinal endoscope to directly identify the bacteria **(Lee JY and Kim N, 2015).** However, invasive techniques including culture, histological inspection, rapid urease test (RUT), and biopsy-based molecular testing are affected by the location, quantity, and size of biopsy specimens **(Gastli et al., 2021)**. In addition, these methods have other limitations including high cost and requiring trained personnel and properly equipped facilities. The invasive biopsy-based approaches are therefore unreliable for monitoring treated patients. Recently, noninvasive tests can be performed on serum, saliva, or urine for antibody detection, on stool or saliva for antigen detection, or breath samples indicating bacterium urease activity **(Kayali et al., 2018)**. These tests are efficacious for diagnosis and screening of *H. pylori* infection with good validation when compared with culture on gastric biopsies and then offer an alternative reliable diagnostic approach for assessment of cure after treatment of infected patients **(Bordin et al., 2021).** In this context, a sensitive and specific noninvasive enzyme-linked immunosorbent assay (ELISA) based on the detection of *H. pylori* circulating antigen (HpCA) in serum samples from *H. pylori*-infected individuals was developed by **Attallah et al., (2004).** The target HpCA has been detected by ELISA with a high degree of efficiency (> 90%). In addition, the target HpCA was detected in CSF samples of patients with meningitis **(Attallah et al., 2007)** and sera of patients with different types of gastrointestinal cancers **(Abdel-Raouf et al., 2014).** However, research on the eradication therapy efficacy has not been conducted and the assessment of a cure after treatment of *H. pylori* infection using serum HpCA remains an elusive goal. In the present study, we aimed to evaluate the reliability of HpCA quantitation in human serum using ELISA for noninvasive assessment of cure after a standard triple therapy regimen of *H. pylori* infection in Egyptian patients.

2. Material and methods

2.1. Study patients

The study population consisted of 134 symptomatic individuals (81 males and 53 females, aged 23 - 68 years) selected from patients referred to outpatient clinics of Gastrointestinal Surgery Center (GISC), Faculty of Medicine, Mansoura University during the period August 2018 to January 2020 and underwent upper gastrointestinal endoscopy and biopsy for dyspepsia. A full medical examination was performed on all participants and full clinical history including special habits, history of present illness, history of previous similar conditions, and family history was recorded. The following criteria were used to determine who would be excluded from the study: 1) use of antimicrobials, proton-pump inhibitors (PPIs), and H_2 blockers within the eight weeks before the endoscopy; 2) history of upper digestive hemorrhages and gastric cancer; and

3) existence of any underlying systemic diseases, such as heart disease, when combined with the use of antiplatelet and anticoagulants. The present research was conducted following the Helsinki declaration and approved by the ethical committee of GISC, and all participants gave their consent to the study following the informed consent regulations of GISC, Mansoura University.

2.2. Blood samples and biopsy specimens

Three mL of blood were obtained from each individual before and after the end of a treatment protocol. The serum was separated from the blood, and aliquot and stored at -70 ^{o}C till screened for HpCAg using ELISA as described later. Four biopsy specimens were taken from the antrum: two for histopathology and histological staining with hematoxylin and eosin (H & E) and Giemsa stain, one for RUT, and one for *H. pylori* culture testing. Biopsies were collected in sterile screw-capped bottles containing 1 mL sterile transfer media or nutrient broth **(Soltesz et al., 1992).** Biopsy specimens for histopathology and histology staining were fixed in 10% phosphate-buffered formalin for the preservation of tissues (preventing autolysis of tissue by endogenous enzymes). The other biopsy specimens subjected to *H. pylori* investigations were placed in 0.9 % isotonic solution (Sterile & Non-pyrogenic normal saline, Lot # 3J 88.3, A. Otsuka Co., Japan).

2.3. Biopsy-based diagnostic methods of *H. pylori* infection

2.3.1. Histopathology and histological staining

The biopsy specimens were embedded in paraffin and serially sectioned at 5 mm. Then, the slides were stained with H & E according to the conventional method at room temperature (**Steven A and Wilson I, 1999)** or with the modified Giemsa stain described by **Gray et al. (1986)**. Stained slides were examined by a histopathologist blinded to the patient's clinical data. Histochemical staining is important for the detection of lesion distribution, degree of gastritis, types of cells, and state of activity. Also, it is valuable in the detection of *H. pylori*, which appeared as curved bacilli at the lumen border.

2.3.2. Rapid urease test

A portion of the ground-homogenized biopsies was inoculated onto a slope of Christensen's urea agar, where it was incubated for 24 hours at 37°C under microaerophilic conditions. Within 24 hours of the specimen being inoculated into the Kimberly-Clark CLO Rapid Urease Test, a color change from yellow to pink was considered positive RUT (**Collee et al., 1996).**

2.3.3. Culture

The biopsy specimen of gastric mucosa was transported to the microbiology laboratory within 2 h. In the laboratory, each biopsy was cultured after homogenization over Columbia blood agar (CM 331, Oxoid, England) supplied with 5% sheep blood and the antibiotic supplements using a tissue homogenizer (Kontes, Vineland, New Jersey, USA) according to **Ansorg et al. (1991).** Plates were incubated at 37 ^{o}C up to 72 h in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2). On agar plates, *H. pylori* colonies show up as tiny, translucent grey colonies that are 0.5–1 mm in diameter. Before a final report is provided, plates are incubated for a total of 96 hours (i.e., 7 days) using gas packs (Campy Pak; Becton Dickinson) in an anaerobic jar if growth is not seen. Bacterial growth was then identified as *H. pylori* based on colony morphology on Gram stain and production of Urease, Catalase, and Oxidase enzymes (**Collee et al., 1996)**.

2.4. Susceptibility testing using the Kirby-Bauer disk diffusion method

After drying, a chocolate blood agar plate (made with 5% horse blood and agar powder) was inoculated with 0.2 mL of *H. pylori* suspension. The dried agar plates were then covered with antibiotic discs **(Jorgensen JH and Turnidge JD, 2007).** The diameter of the inhibitory zones for amoxicillin (18 mm), clarithromycin (30 mm), metronidazole (28 mm), and tetracycline (4 mm) were used to define resistance. Patients carrying drug-resistant strains to

the *H. pylori* eradication study regimen (i.e., resistance to clarithromycin and/or amoxicillin) were excluded from further analysis and subjected to another study regimen. Only patients with a positive H. pylori culture and antibiotic susceptibility result were included in the final analysis.

2.5. Detection and quantification of HpCAg in human serum using ELISA

The target HpCAg was detected and quantified in serum samples using an ELISA kit (ABC Diagnostics, New Damietta, Egypt) according to the manufacturer's instructions. The detection range of HpCAg in serum was 5 to 320 µg/mL and the intra- and inter-assay variations were < 10%. In brief, 100 microliters of standards or serum samples were added into the provided micro-ELISA plate wells and incubated overnight at 4 °C. All standards, blank, and samples were tested in duplicates. After the liquid of each well was removed without washing, a blocking solution was added for 30 min. After washing, a detection antibody specific for HpCAg was added to each well and incubated for 1 h at 37 °C. After free components were washed away, HRP conjugate was added and incubated for 1 h at 37 °C. After extensive washing, the substrate solution is added to each well and incubated for 15 min at 37 °C. Then, the enzyme-substrate reaction is terminated by the addition of Sulphuric acid solution. The color density was measured at a wavelength of 450 nm using a microplate reader (EZ Read 400, Biochrom Ltd, UK). The standard antigen concentrations were used to establish a calibration curve and the assay cut-off level was set at 25 µg/mL. The concentration of HpCAg in each serum sample was calculated from the established calibration curve. The screened sample with concentration ≥ the cut-off (25 µg/mL) is considered HpCAg positive and the sample with HpCAg concentration < 25 µg/mL is considered HpCAg negative.

2.6. Treatment regimen of *H. pylori* infection:

All patients with confirmed *H. pylori* infection i.e. showing positive results by using both microbiological culture and HpCAg-ELISA and showing no resistance to any of the suggested drugs were eligible and subjected to proton pump inhibitor (PPI)-based triple therapy and follow-up. The treatment regimen comprised Lansoprazole (30 mg; twice daily), Clarithromycin (500 mg; twice daily), and Amoxicillin (1000 mg; twice daily) for 14 days **(Paoluzi et al., 2006; Malfertheiner et al., 2017).** Six weeks after the end of eradication treatment, all individuals were referred to the laboratory for repeating HpCAg testing using ELISA to evaluate the outcome of treatment. During this period, all treated patients have not taken proton-pump inhibitors and antibiotics. A negative HpCAg test conducted at least six weeks following the end of the prescribed treatment regimen was considered proof of successful *H. pylori* eradication.

2.7. Statistical analysis

Statistical analyses were performed using SPSS 22, Sydney, NSW, Australia). The Kolmogorov–Smirnov test was used to determine data distribution. The Kolmogorov–Smirnov test was used to determine data distribution. The quantitative data were expressed as Mean ± SD and categorical variables were expressed as frequencies and percentages. Data with non-normal distribution were expressed as Mean ± standard error of the mean (SE) and Median. Fisher's exact test was used to calculate the significance between categorical variables, and Kruskal Wallis and Mann Whitney U test were used to calculate the difference in quantitative variables. Correlations between variables were calculated by Spearman's correlation coefficient. The gold standard for positive in the current study was defined as having at least two positive test results across histology, RUT, and culture, whereas the gold standard for negative was defined as having at least two negative test results throughout the culture to demonstrate the absence of active infection. We calculated the area under the receiver operating characteristic (AUROC) curve, which was expressed as plots of the test sensitivity vs. 1 - specificity, to assess the diagnostic utility of the HpCAg marker

for *H. pylori* infection. The AUROC represents the likelihood that a randomly chosen case has a higher marker value than a randomly chosen control and runs from 0.5 (for a non-informative marker) to 1 (for a perfect marker). Furthermore, the performance characteristics including sensitivity, specificity, and efficiency were calculated from the 2 × 2 table for the HpCAg-ELISA test in comparison with the gold standard results as a reference. All statistical calculations were performed with a significant threshold of 0.05.

3. Results

3.1. Clinicopathological characteristics of the screened study individuals:

The ages of the study individuals follow the normal distribution ($p = 0.07$ i.e., not significant according to the Kolmogorov-Smirnov test of normality) and the peak incidence was in individuals aged ≥ 40 yr. No significant difference ($p > 0.05$) was shown between the mean ages of males (45.76 ± 10.56 yr) and females (43.04 ± 11.01 yr). According to endoscopic examination, the 134 screened individuals were classified into 16 patients with normal gastric mucosa, 98 patients with gastric disorders (90 patients with gastritis and 8 patients with a gastric ulcer), and 20 patients with duodenal disorders (15 patients with duodenitis and 5 patients with the duodenal ulcer). Gastritis represents the major common upper gastrointestinal disorder (67%) by endoscopy among screened patients. The screened individuals were classified histopathologically into four categories: normal gastric mucosa (NGM, n = 24), chronic superficial gastritis (CSG, n = 44), chronic deep gastritis (CDG, n = 35), chronic atrophic gastritis (CAG, n = 24) and intestinal metaplasia (IM, n = 7). CSG represents the major pathological findings (33 %) among screened patients. In addition, the severity of gastritis for all investigated patients were classified as no gastritis (n = 16, 12 %), mild gastritis (n = 29, 22 %), moderate gastritis (n = 48, 36 %) and severe gastritis (n = 41, 30 %). Moderate gastritis represents the major common degree among the screened patients.

3.2. Screening of *H. pylori* infection using invasive biopsy-based techniques:

A total of 90 (67.2 %) out of 134 screened individuals showed positive culture testing results whereas 44 (32.8 %) individuals showed negative culture testing results. A total of 87 (65 %) out of 134 screened individuals showed positive RUT results and 47 (35 %) showed negative RUT results. A total of 71 (53 %) out of 134 screened individuals showed positive *H. pylori* Giemsa staining results and 63 (47 %) showed negative *H. pylori* staining. According to the gold standard, 90 (67.2 %) out of 134 screened individuals have been diagnosed *H. pylori*-infected whereas 44 (32.8 %) individuals were diagnosed free of *H. pylori* infection. Antibiotic sensitivity testing was performed for all 90 gastric isolates showing positive culture testing results of *H. pylori*. A total of 28 (31%) out of 90 isolates showed resistance to one or both of these two antibiotics in a study treatment regimen. Four isolates (4 %) showed resistance to Amoxcyclin, 16 isolates (18 %) showed resistance to Clarithromycin and 8 isolates (9 %) showed resistance to both Clarithromycin and Amoxcyclin. All individuals showing drug resistance were excluded and subjected to another study treatment protocol. The remaining 62 (69 %) out of 90 isolates showed sensitivity to these two antibiotics and hence their patients were eligible for inclusion in the treatment protocol.

3.3. Non-invasive detection and validation of serum HpCAg using ELISA:

A total of 91 (68 %) out of 134 screened individuals showed HpCAg concentration ≥ 25 µg/mL and considered HpCAg positive whereas 43 (32 %) showed HpCAg < 25 µg/mL and considered HpCAg negative. The diagnostic potential of the HpCAg-ELISA test in comparison with gold standard results in 134 Egyptian patients was investigated using ROC curve analysis, Figure 1. The area under the ROC curve equals 0.932 indicating the high diagnostic potential of the ELISA test for the detection of *H. pylori* infection. The

performance characteristics of the HpCAg-ELISA test in comparison with gold standard results showed a sensitivity of 94 %, specificity of 88 %, and total efficiency of 92 %, Table 1. According to the Kolmogorov-Smirnov test of normality, the HpCAg concentrations of 134 study individuals did not follow the normal distribution ($p = 0.0001$) and its data were characterized by Mean ± SE, Median, and Interquartile Range in statistical analyses. The median (Interquartile range) HpCAg concentration in the sera of screened individuals with confirmed *H. pylori* infection was 90.5 (30) was extremely higher than that of 15 (9) in the sera of screened individuals without *H. pylori* infection. A highly significant difference ($p < 0.001$) was shown between the mean serum concentration (± SE) of HpCAg in screened individuals with *H. pylori* infection (86.83 ± 2.86 □g/mL) and that of screened individuals without *H. pylori* infection (22.45 ± 3.06 □g/mL), Figure 2. In addition, no significant correlations are shown between HpCAg concentration with sex (Spearman's rho = - 0.15, $p > 0.05$) and with age (Spearman's rho = - 0.08, $p > 0.05$) of the 134 screened individuals. The presence of HpCAg was significantly ($p < 0.01$) associated with endoscopic findings including gastric disorders (G and GU) and duodenum disorders (D and DU) in comparison with non-infected screened individuals, Figure 3A. Also, the presence of HpCAg was significantly ($p < 0.01$) associated with the different pathological disorders including CSG, CDG, CAG, and IM (Figure 3B) as well as with the severity of gastritis in comparison with non-infected individuals (Figure 3C).

3.4. Follow-up of *H. pylori* infection after Treatment using HpCAg-ELISA:

Only 50 out of 62 treated patients provide serum samples for the detection of HpCAg using ELISA at the end of the treatment protocol. Three female patients could not receive therapy because of other comorbid situations that appeared during follow-up and 9 patients did not admit at a specified time for control. Consequently, the data of these 12 individuals were excluded from further analysis. Before treatment, the mean of HpCAg concentrations (± SE) of 50 treated individuals was 92.08 ± 3.08 µg/mL and after treatment, it was 24.00 ± 2.34 µg/mL. In addition, a highly significant decrease ($p < 0.001$) was shown in HpCAg concentrations after treatment (Figure 4). No significant differences were shown between the mean HpCAg concentrations of 24 treated males and 26 treated females after treatment, Table 2. After treatment, 39 (78 %) out of 50 treated individuals showed HpCAg negative results (i.e. responders to treatment) and 11 (22 %) showed HpCAg positive results (i.e. non-responders to treatment). The HpCAg concentrations (15.77 ± 0.58 µg/mL) of responders were significantly decreased ($p < 0.005$) in comparison with their HpCAg concentrations before treatment (87.90 ± 3.35 µg/mL). Also, the HpCAg concentrations (53.18 ± 2.91) of non-responders were significantly decreased ($p < 0.005$) in comparison with their HpCAg concentrations before treatment (106.91 ± 5.69 µg/mL), Figure 5. Before treatment, signs, and symptoms of *H. pylori*, infection was recorded for all 50 treated individuals. At the end of a treatment protocol, no signs and symptoms of *H. pylori* infection were recorded for all 39 responders showing HpCAg negative results and for 4 out of 11 non-responders showing HpCAg positive results. Only 7 out of 11 non-responders still have abdominal discomfort as well as dyspeptic symptoms associated with *H. pylori* infection. The endoscopy and biopsy-based diagnostic methods were only repeated for all 11 non-responders. The endoscopy and histopathology indicated no signs of severe gastritis and ulcers. In addition, histology, rapid urease test, and culture showed *H. pylori*-negative results for all non-responders.

Table 1. Performance characteristics of HpCAg-ELISA test for diagnosis of *H. pylori* infection among 134 screened individuals in comparison with culture, RUT, and histology methods.

The gold standard for diagnosis	HpCAg ELISA*		Total
	Positive	Negative	
Positive	85 (TP)	5 (FN)	**90**
Negative	6 (FP)	38 (TN)	**44**
Total	**91**	**43**	**134**

* Sensitivity (%) = TP / (TP + FN) = 85 / (85 + 5) × 100 = 94 %.
Specificity (%) = TN / (TN + FP) = 38 / (38 + 6) × 100 = 88 %.
Efficiency (%) = (TP + TN) / Total = (85 + 38) /134 × 100 = 92 %.

Table 2. The HpCAg concentrations using ELISA concerning the gender of treated individuals before and after treatment.

Gender**	No.	HpCAg Conc. (μg/mL)				P value*
		Before Treatment		After Treatment		
		Mean	SE	Mean	SE	
Male	**24**	94.25	4.38	26.75	3.64	< **0.001**
Female	**26**	90.08	4.38	21.46	2.98	< **0.001**
Total	**50**	92.08	3.08	24.00	2.34	< **0.001**

* A highly significant decrease (P < 0.001) was shown in HpCAg concentrations after treatment in comparison with HpCAg concentrations before treatment.

** No significant difference was shown between HpCAg concentrations of males and females either before or after treatment.

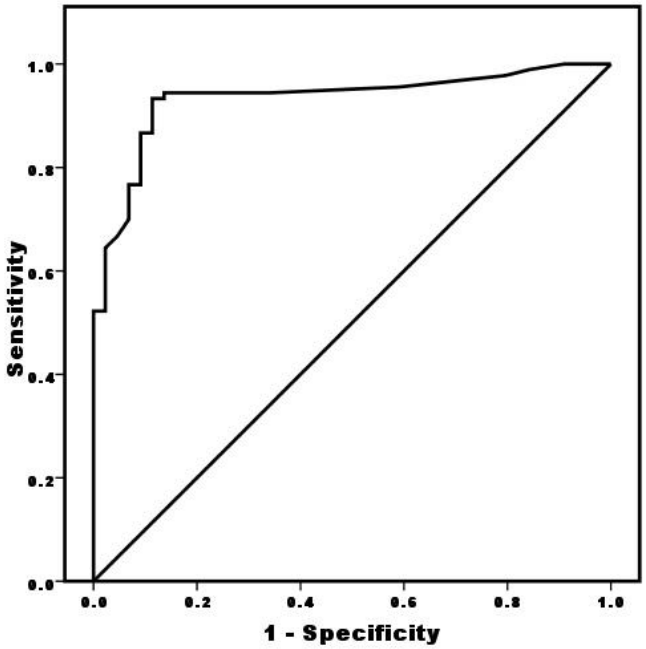

Figure 1. ROC curve analysis of HpCAg ELISA in comparison with a gold standard for diagnosis of *H. pylori* infection of screened individuals. The area under ROC equals 0.932 which indicates high-performance characteristics of the HpCAg ELISA test.

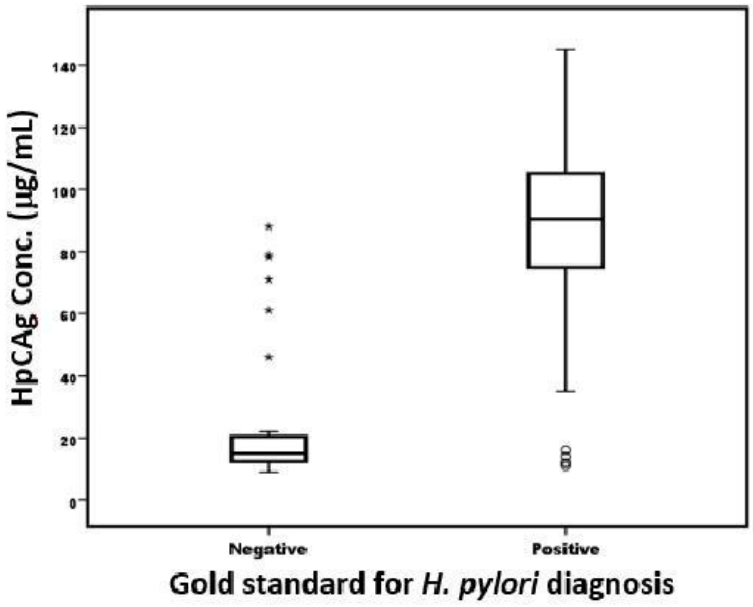

Figure 2. Boxplot analysis of HpCAg concentrations concerning results of a gold standard for diagnosis of *H. pylori* infection of 134 screened individuals. A highly significant difference ($p < 0.001$) was shown between the mean serum concentration of HpCAg in screened individuals with *H. pylori* infection and that of screened individuals without *H. pylori* infection**.**

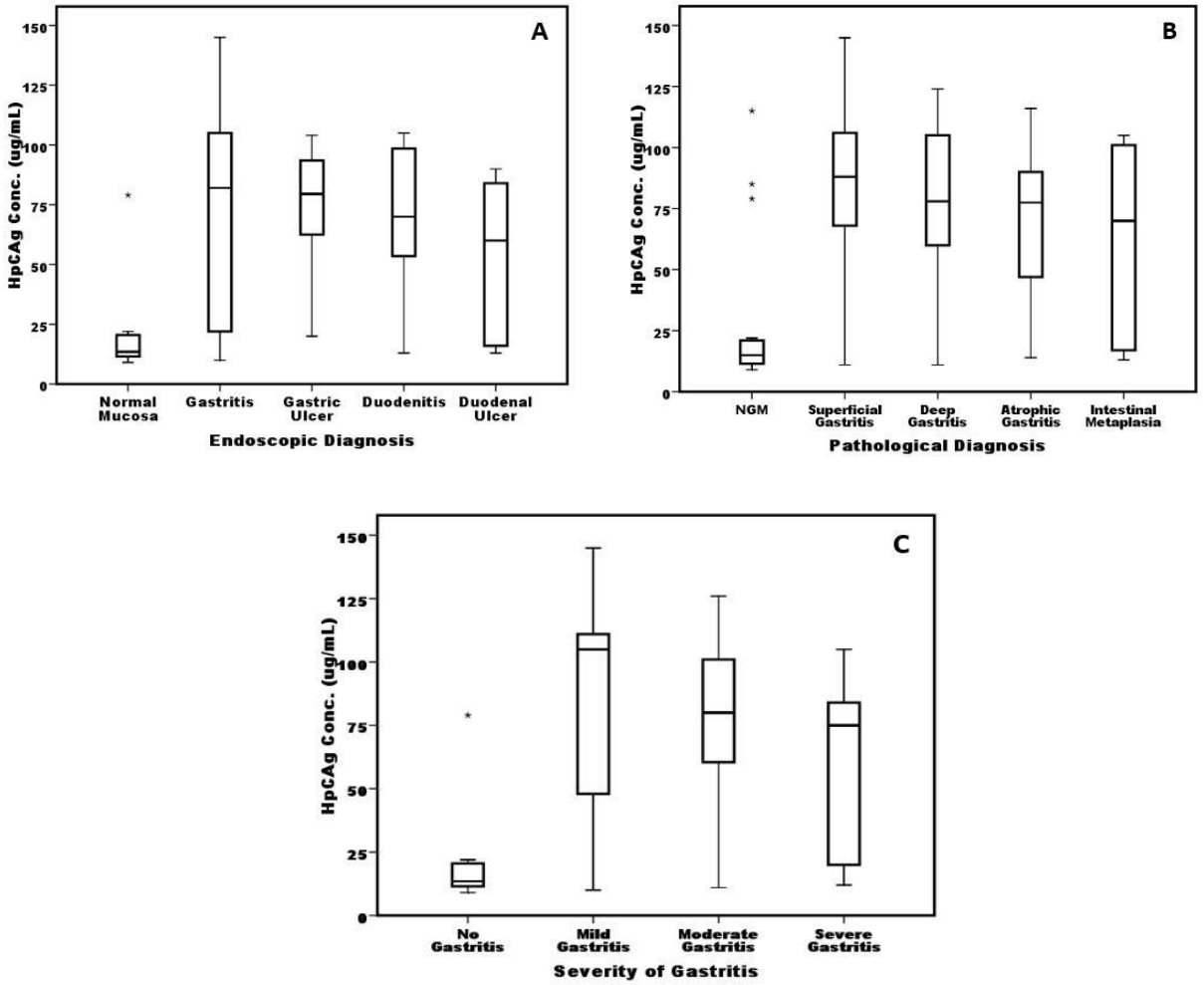

Figure 3. Boxplot analysis of HpCAg serum concentrations (µg/mL) based on: A) Endoscopic findings, B) Pathological findings C) and Severity of gastritis of 134 screened individuals.

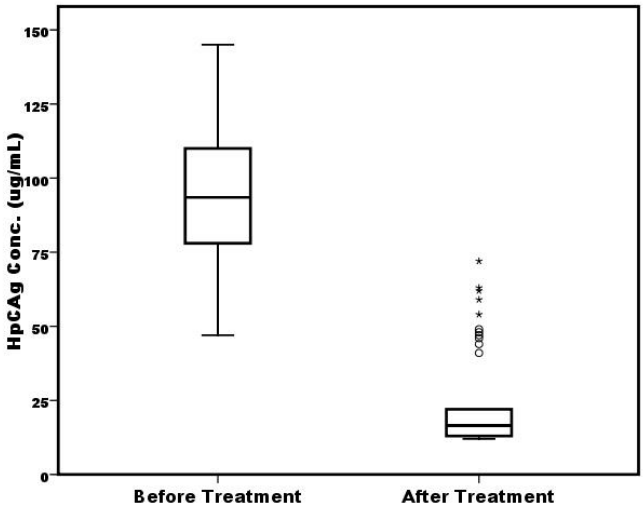

Figure 4. Boxplot analysis of HpCAg concentrations before and after treatment. A significant decrease was shown in HpCAg concentrations after treatment in comparison with HpCAg concentrations before treatment ($p < 0.001$).

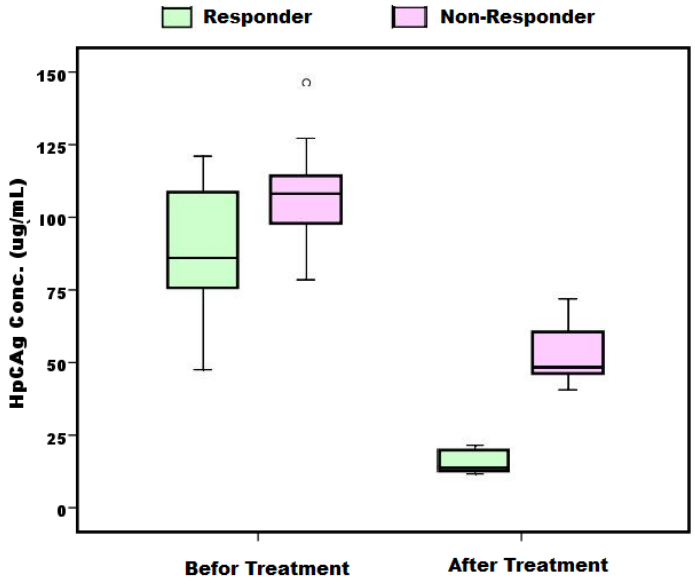

Figure 5. Boxplot analysis of HpCAg concentrations of responders and non-responders to treatment. A significant decrease was shown in HpCAg concentrations after treatment in comparison with HpCAg concentrations before treatment ($p < 0.001$) of both responders and non-responders. However, the median HpCAg concentration of 11 non-responders is higher than that of 39 responders either before or after treatment.

4. Discussion

Patients chronically infected with *H. pylori* were estimated to have 20 times increased risk of developing gastric cancer than uninfected patients **(Kim et al., 2011; Yang et al., 2015; Cubiella et al., 2021).** Clinicians would require a reproducible test to screen patients for the presence of this bacterium, and if a patient tested positive for *H. pylori*, to track the efficacy of eradication therapy. **(Krüttgen et al., 2012).** Furthermore, the need for a reliable and sufficiently accurate noninvasive test for screening and follow-up of *H. pylori*-infected individuals has been the most important limit to their spread. To reduce the expense, inconvenience, and discomfort related to invasive procedures, non-invasive techniques are favored over invasive tests for the diagnosis of *H. pylori* infection. Noninvasive *H. pylori* testing can be used successfully for pre-endoscopic screening of children, young adults, and adults referred to a gastroenterology service to investigate dyspepsia **(Holmes et al., 2010)** as well as for therapeutic monitoring following eradication therapy **(Gisbert JP, 2020)**, in addition to facilitating epidemiological studies **(Douraghi et al., 2013).** In the present study, we aimed to investigate the reliability of a noninvasive ELISA based on the detection of HpCAg in serum for screening of *H. pylori* infection and evaluate its usefulness for follow-up of *H. pylori*-infected Egyptians after clarithromycin-based triple therapy. Most (67%) of our 134 screened individuals were with endoscopic gastritis with a moderate degree of severity in 36% of them. In addition, CSG represents the major pathological findings among screened patients (33 %). *H. pylori* infection is frequently linked to several upper gastrointestinal diseases, and research

suggests that about 50% of people worldwide have the infection, although >80% of them do not experience any symptoms. **(Testerman TL and Morris J, 2014).** However, the *H. pylori* infection was confirmed using a gold standard based on the results of culture testing, RUT, and Giemsa staining-based histology. According to the applied gold standard for diagnosis, 67.2 % out of screened individuals were diagnosed with *H. pylori*-infected whereas 22.8 % of individuals were diagnosed free of *H. pylori* infection. We validated the performance characteristics of the noninvasive HpCAg-ELISA test among 134 adult Egyptian patients. The diagnostic potential of the HpCAg-ELISA test in comparison with gold standard results in 134 screened Egyptian patients was investigated first using the area under the ROC curve analysis. The AUROC curve equals 0.932 indicating the high diagnostic potential of the ELISA test for the detection of *H. pylori* infection **(Nahm, 2022).** Then, the performance characteristics of the HpCAg-ELISA test were evaluated in comparison with the results of the study's gold standard for diagnosis. A sensitivity of 94 %, specificity of 88 %, and a total efficiency of 92 % were shown for the HpCAg-ELISA test. These high degrees of performance characteristics are like that of the original study performed by **Attallah et al. (2004)** and comparable to that of the first-line noninvasive techniques; non-radioactive ^{13}C-labeled urea breath test; UBT; for determining active *H. pylori* infection **(Boltin et al., 2018)** and Stool antigen testing **(Calvet et al., 2013).** The UBT depends on the viable bacterial usage of ^{13}C and is considered the most accurate noninvasive test with a sensitivity of 88-95% and specificity of 95%-100%, Although UBT is also reliable in post-treatment follow-up **(Tonkic et al., 2018),** it is expensive, difficult to perform and its results affected by stomach conditions **(Gisbert JP and Pajares JM, 2004).** On the other hand, although its sensitivity and specificity of > 95% and continuous optimization of assay conditions **(Qiu et al 2021; Kakiuchi et al., 2022)**, stool antigen testing may be less acceptable to many individuals for several reasons **(Lario et al., 2016)**. Because *H. pylori*-specific antigens in the stool samples are diluted, the accuracy of stool antigen testing is decreased when the stool samples are unformed or watery (**Zhou et al., 2014)**. The results of stool antigen testing can also be impacted by temperature and the time between collecting and measuring a stool sample (**Shimoyama T, 2013)**. In addition, the heterogeneity in the type of test kits used (i.e. enzyme immunoassay versus immunochromatographic assay and monoclonal versus polyclonal antibodies), and the potential errors in sample preparations from different laboratories were also indicated as limitations in several diagnostic accuracy studies **(El-Shabrawi et al., 2018; Alboraie et al., 2019).** As a result, our study's findings support the notion that the HpCAg-ELISA test may reliably diagnose *H. pylori* infection and that it can be used to screen dyspeptic patients before referring them for endoscopy. Serum antigen detection also provides a trustworthy alternative diagnostic test for *H. pylori*. The serum antigen-based test is a particularly alluring substitute for the stool antigen-based test in large-scale or even small studies of *H. pylori* because serum sampling may offer a higher response rate than stool sampling in volunteer research **(Attallah et al., 2022).** Even though *H. pylori* infection is a public health issue that frequently has catastrophic side effects, especially in developing countries like Egypt **(Hooi et al., 2017)**, only a few investigations were conducted, and they discovered that both adults and children had high rates of infection of around 70% **(Sayed et al., 2007; Sabah et al., 2015; Galal et al., 2019).** Here, a nearly equivalent or slightly lower prevalence rate (68 %) of *H. pylori* infection was shown using HpCAg-ELISA (i.e. showing HpCAg concentration ≥ 25 µg/mL) among screened individuals without significant correlations between HpCAg concentrations and both sex and age.

However, more epidemiological studies are needed to cover most Egyptian Districts with special attention to socioeconomic status, other associated organisms, other associated diseases, environmental factors exploration, and genetic factors. In the present study, the presence of HpCAg was significantly ($p < 0.01$) associated with endoscopic findings including gastric disorders (G and GU) and duodenum disorders (D and DU), and with the different pathological disorders including CSG, CDG, CAG, and IM as well as with the severity of gastritis. Chronic gastritis has been linked to *H. pylori* as a cause **(Bittencourt et al., 2006; Potamitis GS and Axon AT, 2015).** It is advised that all patients with confirmed *H. pylori* infections receive the proper antimicrobial care **(Georgopoulos et al., 2013, O'Connor et al., 2015)** and the effective *H. pylori* eradication treatment should achieve a cure rate higher than 90 % **(Gisbert JP, 2020).** Many therapeutic regimens have been proposed **(Fallone et al 2016),** however, the results of treatment are controversial, and the ideal therapy does not still exist **(Malfertheiner et al., 2017; Chey et al., 2017).** Recently, *H. pylori* eradication treatment is indicated regardless of the associated clinical condition **(Pimentel-Nunes et al., 2019).** The promising results and the association with clinicopathological characteristics encourage us to further investigate the use of serum HpCAg detection using ELISA for noninvasive follow-up of infected patients after treatment for the first time. For patients with *H. pylori* positivity, clarithromycin-based regimens are frequently utilized as the first line of treatment **(Ierardi et al., 2017).** The chosen first-line treatment for the eradication of *H. pylori* infection in Egypt is still 14 days of clarithromycin-based triple therapy, despite numerous studies confirming the existence of resistance **(Alboraie et al., 2019).** All patients with confirmed H. pylori infection in this study, defined as having positive results by both microbiological culture and HpCAg-ELISA, were eligible and received triple therapy based on proton pump inhibitors (PPI) and followed up. The use of a PPI is preferable to reduce infection-related symptoms and adverse effects from concomitant medications and increase the possibility of *H. pylori* eradication **(Yuan et al., 2013).** In addition, we performed antibiotic susceptibility testing to exclude patients infected with *H. pylori* who have probably become resistant to any antibiotics of the first-line triple dose regimen. To enable the administration of the typical clarithromycin-based triple therapy to patients with *H. pylori* clarithromycin-susceptible strain in regions with high overall clarithromycin resistance as well as to minimize the development of antimicrobial resistance, antibiotic susceptibility testing before first-line therapy is advised **(Arslan et al., 2017; Dang BN and Graham DY, 2017).** A total of 31% out of 90 infected individuals showed drug resistance to at least one of two antibiotics included in the current study treatment regimen and consequently were excluded from further analysis. However, these individuals infected with resistant strains of *H. pylori* were subjected to another study treatment protocol. The remaining 69 % out of 90 isolates showed sensitivity to these two antibiotics and hence their patients were eligible for inclusion in the treatment protocol. After treatment, a highly significant decrease ($p < 0.001$) was shown in HpCAg concentrations for all treated individuals without significant differences between males and females. In addition, 78 % of treated individuals showed HpCAg-ELISA negative results and were considered responders to the treatment regimen and the remaining 22 % showed HpCAg-ELISA positive results and were considered non-responders to the treatment regimen. It is hypothesized that the presence of false positives after the eradication therapy could be linked to the physiological elimination of antigens from gastric cells containing *H. pylori* without real infectious capacity (**Kayali et al., 2018)**. The eradication rate (78 %) achieved in the present study using standard Clarithromycin

triple therapy is higher than that reported in other studies **(Afifi et al., 2020).** This may be due to the exclusion of all patients showing antibiotic resistance in *H. pylori* susceptibility testing. Notably, the current literature also shows that sensitivity-guided therapy is more effective than concomitant therapy as well **(Sugimoto M and Furuta T, 2014; Zaki et al., 2016; Elrakeeb et al., 2021).** Before treatment, the signs and symptoms of *H. pylori* infection were recorded for all study-treated patients. At the end of the treatment protocol, no signs and symptoms of *H. pylori* infection were recorded for all responders and 4 non-responders. However, 7 non-responders still have abdominal discomfort as well as dyspeptic symptoms associated with *H. pylori* infection. These results confirm the association between HpCAg-ELISA results and clinical investigations of treated individuals. However, the endoscopy and biopsy-based diagnostic methods were repeated for all non-responders to confirm the clinical investigation and obviate association with HpCAg ELISA results. The endoscopy and histopathology indicated no signs of severe gastritis and ulcers associated with persistent *H. pylori* infection. In addition, histology, RUT, and culture showed *H. pylori*-negative results for all non-responders confirming the significant decrease of HpCAg concentrations in their sera after treatment. The persistence of the bacteria in the stomach is dependent on mononuclear phagocytes, and these cells are key controllers of the mucosal immune response during *H. pylori* infection **(Pagliari et al., 2017)**. Recent research has revealed that rather than aiding in bacterial clearance, phagocytic cells during an *H. pylori* infection increase high *H. pylori* burdens **(Viladomiu et al., 2017)**; in accordance, it was expected by computer modeling of immunological responses to *H. pylori* that macrophages are the primary controllers of the mucosal immune response. **(Carbo et al., 2013).** However, further investigation is required to draw the kinetics of target *H. pylori* antigen clearance from blood after treatment. In conclusion, HpCAg detection is a reproducible approach for noninvasive diagnosis and screening of *H. pylori* infection. Furthermore, this test can be used to demonstrate that gastric *H. pylori* has been eradicated, especially in developing countries. However, to further confirm the results of the HpCAg-ELISA test, an additional large population and multi-central follow-up studies are still required.

Author contributions

HI, and MAW study conception and design, writing the original draft. MAW performed medical history and endoscopy. HRF, AMA, and HI performed methodology and collection of data. HI, AMA and MAS performed analysis and interpretation of results. The final version of the text was reviewed and approved by all authors.

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Availability of data

The corresponding author can provide the original data upon request (Email: himosman@mu.edu.eg).

Conflict of interest:

The authors declare that they have no conflict of interest.

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