## Original Research Article

# Evaluation of serological test in the diagnosis of *Helicobacter pylori* and risk factors associated with the infection

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**ABSTRACT**

**Background:** Serological testing has been widely used for the diagnosis of *H. pylori*. This study aimed to evaluate the serological test and to determine the sensitivity and specificity of the test in the diagnosis of *H. pylori*. The study also aimed to address if there are risk factors like blood grouping, Smoking, Age, gender, and residence of the patients associated with *H. pylori* infection.

**Methods:** A prospective cross-sectional study was performed among 100 symptomatic patients attending Dr. Suliman dispensary, Elnehoud city in west Kordofan state-Sudan, from March to September 2016. *H. pylori* were detected on plasma by using Healgen immunochromatography test cards from Xiamen Boson Biotech Co., Ltd (China), and identified from a stool by using monoclonal antigen detection from the same trademarked company. Data for the risk factors associated with the infection were assessed in a participant interview.

**Results:** The serological test showed significant differences when compared to the stool antigen test p-value = 0.000. The statistical analysis showed moderate sensitivity and high specificity of the serological test compared to the stool antigen detection test. The study also showed that smoking [odds ratio (OR): 1.20, 95% confidence interval (CI): (1.24-4.02) and blood grouping (OR: 1.10, 95% CI: (1.08-1.60) were risk factors for *H. pylori* infection.

**Conclusions:** The serological test showed high specificity and moderate sensitivity in comparison to the stool antigen test. The increased risk of *H. pylori* infection associated with smoking and blood grouping.

**Keywords:** *H. pylori,* Risk factors, Serological test, Sensitivity, Specificity

## INTRODUCTION

*Helicobacter pylori* is a curved gram-negative bacillus. *H. pylori* infection has been etiologically associated with several pathogenic conditions of the stomach, including Gastritis, peptic ulcer, gastric carcinoma, and mucus associated lymphoid tissue (MALT) lymphoma.[1]

Infection with *H. pylori* is a worldwide health problem, especially in developing countries; more than 80% of the population infected with *H. pylori*.[2] In Sudan, the prevalence of the disease estimated to be 65.8% of the population.[3]

Transmission of *H. pylori* is via feco-oral, oro-oral, gastro-oral, gastro-gastric, and person-to-person routes.[4] The risk factors for the transfer of *H. pylori* include race, socioeconomic status, residing in a rural area, age, poor sanitary conditions, overcrowding, poor diet, inadequate water supply, and lower educational level of mothers.[5]

Laboratory testing for *H. pylori* infection is a crucial part of the diagnosis, although many different diagnostic test methods, both invasive and noninvasive, are available.[6] Serology test is used widely in the determination of the infection because it is a cheap, easy to perform and can be implemented in small laboratories, the test involves detection of patients' blood, plasma or serum for IgG and IgA antibodies.[7] On the other hand, the *H. pylori* stool antigen test has been put in the market as an optional technique because of its reliability and simplicity. It's an excellent diagnostic accuracy in pre- and post-treatment cases of *H. pylori* infections.[8]

Several factors have been associated with the aggressiveness of *H. pylori* and hence implicated in epithelial damage, including the virulent East Asian *CagA* genotype, environmental, and dietary factors.[9] Recently, some researchers have also proposed that certain nutritional elements such as chili peppers and garlic play a protective role against *H. pylori* infection.[10]

Despite the extensive use of serological tests in the study area as a diagnostic test for the *H. pylori* infection, no previous study performed to evaluate the test in this area. Therefore, this study aimed to assess the serological test and to determine the sensitivity and specificity of the test in the diagnosis of *Helicobacter pylori* in comparison to the stool antigen detection method. The study also aimed to address if there are risk factors like blood grouping, Smoking, Age, gender, and residences associated with *H. pylori* in western Sudan.

## METHODS

The study included 100 symptomatic *H.Pylori* infected patients from both genders; their ages ranged from 20 and 60-years old. All patients have attended Dr. Suliman dispensary, Elnehoud city in western Kordofan state, Sudan, during March to September 2016. Participants excluded from the study if they used antibiotics, proton pump inhibitor a month before examination or recently diagnosed, or under treatment with *H. pylori* infections, those who were pregnant and those who underwent gastrectomy also excluded. Study approved by the Ethical Committee of the Ministry of Health at Elnehoud city-Sudan in addition to written informed consent from all patients who were obtained before sample collection. Demographic and clinical data recorded from each patient before sample collection.

A total of 2 ml of venous blood collected into plain blood container, then serum separated by centrifugation to detect *H. pylori* antibody using Healgen immunochromatography test cards from Xiamen Boson Biotech Co., Ltd (China). Besides, Fresh stool samples collected from each patient into a spoon-cover and outer-labeled stool container for antigen detection. Using wood sticks, a small portion of the stool sample was transferred into the buffer provided by Biotech and Healgen and incubated for 2 minutes. Then 2 to 3 drops of the mixture were poured in the hole of the ICT of *H. pylori* stool antigen detection.

Data concerning the risk factors for *H. pylori* infection collected in a structured questionnaire using a one-on-one participant interview to be analyzed.

### Statistical analysis

Data collected and analyzed using SPSS version 21. A Chi-square test was used to compare frequency positivity for the serology and stool Ag test results. The sensitivity and specificity, were determined. Regression models were established to examining the risk factors (age, gender, blood group, residence, and smoking) associated with developing *H. pylori* infections. Statistical significance was considered when p-value equal to or below 0.05.

## RESULTS

The prevalence rate of *H. pylori* infection was 48%(48/100) by serological test and 28% (28/100) by stool antigen test. Serological test results showed significantly higher positivity for *H. pylori* compared to stool antigen detection, p-value = 0.000, as shown in (Table 1).

**Table1: Frequency of stool antigen and serum antibody for *H. pylori* detection.**

| The result | ICT | | p-value |
|---|---|---|---|
| | Stool Antigen | Serum Antibody | |
| Positive | 28 (28%) | 48 (48%) | 0.000 |
| Negative | 72 (72%) | 52 (52%) | |
| Total | 100 (100%) | 100 (100%) | |

The sensitivity and specificity of the serological test in comparison to a stool antigen test was detected by applying the statistical equations.

$$Sensitivity = \frac{True\ positive}{True\ Positive + False\ Negative} \times 100\%$$

$$Sensitivity = \frac{28}{28+20} \times 100\% = 58\%$$

$$Specificity = \frac{True\ Negative}{True\ negative + False\ Positive} \times 100\%$$

$$Specificity = \frac{72}{72+20} \times 100\% = 78.2\%$$

Data concerning the risk factors for *H. pylori* infection was collected from a structured questionnaire. The results showed 56% were males, and 44% were females. 64% of them were from rural areas, while the rest 36% were from urban areas. Their age ranged from 20 to 60 years old. 42%(42/100) was a regular smoker. Most of the patients

were group O positive 68%(68/100), while 12%(12/100) were group A Positive and group B positive.

The Regression models showed that Smoking [odds ratio (OR): 1.20, 95% confidence interval (CI): 1.24-4.02] and blood grouping (OR: 1.10, 95% CI: 1.08-1.60) were risk factors for *H. pylori* infection. While Resident, age, and gender were not associated with the risk factors of *H. pylori* infection, as shown in (Table2).

**Table 2: Possible studied risk factor for *H. pylori* infection.**

| Characters No=100 | Pylori No= 100 | | p Value | Multivariate analysis | | p Value |
|---|---|---|---|---|---|---|
| | **Present** | **Absent** | | **Odds ratio** | **95% confidence interval** | |
| **Smoking** | | | | | | |
| Yes(42) | 36(86%) | 6(14%) | 0.000 | 1.20 | 1.24-4.02 | 0.000 |
| No(58) | 12(20%) | 46(80%) | | 0.95 | 0.49-2.24 | 0.800 |
| **Residence** | | | | | | |
| Rural area(64) | 29(45%) | 35(55%) | 0.002 | 1.10 | 0.870-1.211 | 0.14 |
| Urban area (36) | 16(44%) | 20(56%) | | 1.4 | 0.549-2.232 | 0.91 |
| **Gender** | | | | | | |
| Male (56) | 28(50%) | 28(50%) | 0.08 | 1.19 | 0.930-1.423 | 0.73 |
| Female(44) | 24(55%) | 20(45%) | | 1.80 | 0.81-3.99 | 0.15 |
| **Blood grouping** | | | | | | |
| Group O positive (68) | 59(87%) | 9(13%) | | 1.23 | 1.22-2.23 | 0.000 |
| Group A Positive (12) | 12(100%) | 0(0%) | | 1.10 | 2.11-4.02 | 0.000 |
| Group A Negative (4) | 1(25%) | 3(75%) | 0.04 | 1.85 | 0.99-2.07 | 0.860 |
| Group B positive (12) | 8(67%) | 4(33%) | | 1.10 | 1.08-1.60 | 0.000 |
| Group AB positive (4) | 2 (50%) | 2(50%) | | 1.75 | 0.950-2.225 | 0.070 |
| **Age Group** | | | | | | |
| 20-30(32) | 18(56%) | 14(44%) | | 0.60 | 0.28-1.55 | 0.320 |
| 31-40(28) | 15 (54%) | 13(46%) | 0.07 | 1.33 | 0.956-1.120 | 0.10 |
| 41-50(16) | 9(56%) | 7(44%) | | 1.09 | 1.11-1.31 | 0.340 |
| 51-60(24) | 20(83%) | 4(17%) | | 0.73 | 0.44-195 | 0.400 |

The ASC was more in males bilaterally than in females. The difference was statistically significant on right side (P = 0.00). The ASS was more in females than in males on right and vice versa on the left side.

**DISCUSSION**

*Helicobacter pylori* (*H. pylori*) infection is one of the most common chronic diseases in the world.[11] Different methods are available for investigating of *H. pylori* infection.[12] The present study designed to compare the serological test and stool antigen test in the diagnosis of *H. pylori*. Positive *H. pylori* were detected in 48(48%) patients by serological test, while 28(28%) patients only showed positive by stool antigen test. These results were higher than those reported by Naji et al, in Yemen, and lower than those reported in Eastern Sudan by Abdallah et al.[13,14] These differences might be due to differences in ethnic background and age groups of the target populations.

The serological diagnostic tests showed high specificity and moderate sensitivity when compared to stool antigen tests. The study result agrees with Mohammed Hasosah, who reported serological test sensitivity and specificity of (50.9% and 77.9%), respectively.[15] In industrialized countries, where the prevalence of *H. pylori* infection is low, the reported sensitivity of serology is 60%.[16]

Many risk factors were associated with *H. pylori* infection. In this study, blood grouping was significantly associated with *H. pylori* infection. These results reinforce the results of previous studies in Figueria and Kurdistan reported by Jaff, 2011 and Macrolopez et al, respectively.[17,18] Findings of the current study support the epidemiological view of greater susceptibility of blood group (O) to *H. pylori* infection in which H antigen represents an essential receptor for *H. pylori* adherence Dickey et al.[19]

The second risk factor for developing of *H. pylori* infection in our study; is the smoking. Smoking may stimulate the production of pepsinogen and decreases mucous production, leading to more pathology. These results were similar to those reported by Kanby et al, and different from those indicated by Ogihara et al, in Japan

which showed that smoking has a negative association with *H. pylori* infection.[20,21] These variations could be attributed to the fact that the previous investigator used a reasonably large sample size (8837) patients.

Although *H. pylori* infection was most commonly observed in adults with an average age of 40 years, no association was found between the age group and *H. pylori* infection in the present study, this finding in accordance with the research done by Irigrácin Lima, et al.[22]

The association between an increased risk of *H. pylori* infection with gender and residence in the rural area was not statistically significant. Our findings agreed with Irigrácin Lima et al.[22]

*H. pylori* can affect all the ages, and if not diagnosed early, they may develop a severe complication. So, early diagnosis is crucial for an excellent therapeutic option. Another essential fact supporting the needs for evaluation of the serological test, the test is a rapid, simple, and cheap method for *H. pylori* diagnosis implies the test as the only option, especially, in a limited laboratory set up countries where an invasive technique is not available.

## CONCLUSION

The present study showed high specificity and moderate sensitivity of serological tests when compared to stool antigen tests. Findings of positive results in both serum antibody and stool antigen method were relatively highly among (O) positive blood group individuals. Smoking was shown to be an essential risk factor for *Helicobacter pylori* infection. We need more in-depth researches and investigations to correctly generalize rapid and accurate tests in determining *H. pylori,* especially in developing countries.

## ACKNOWLEDGEMENTS

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