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Assessment of Serological Tests for Diagnosis of *H. pylori* Infection among Dyspeptic Patients, Gezira State, Sudan

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Abstract: Background: *H. pylori is one of the most common damaging human pathogen, it identifies as common agent of gastroenteritis infections causes more hard GIT symptoms and potential complications like stomach cancer*. Prevalence of H. pylori in Africa was reported about 79.1%, most likely due to socioeconomic factors. *Several techniques have been developed to give the more reliable results for patient's administration and to insure that the organism was eliminated flowing treatment*.
Objectives: *Many laboratories in Gezira State, Sudan using serological tests to screen and diagnosis the H. pylori through antigen and anti H. pylori antibodies. There for this study was conducted in adult dyspeptic patients to evaluate ELISA and ICT in diagnosis of symptomatic H. pylori infections and comparing with PCR for detection of 16s rRNA gene.*
Method: *Descriptive cross-sectional study was conducted on selected 102 adult dyspeptic patients their age ranged between (20 – 70 years) attending Gezira Center for G.I.T Endoscopy and Laparoscopic Surgery during 2016 – 2019. Stool, venous blood and Antrum biopsy samples were collected from each participants. ICT was used to screen the stool antigen and serum IgG antibody. ELISA technique was performed to investigate serum IgG and IgA. 16s rRNA gene was detected in antrum biopsy sample using PCR. The data of study group was collected by constructed questionnaire and analyzed by SPSS program (V. 20).*
Results: *Epigastric pain (74.0%) was most common clinical finding among patients. According to the PCR method out of 102 samples 53 (51.9%) were positive for H. pylori and 49 (48.1%) were negative. 31 (30.4%) of patients were positive IgA by ELISA with sensitivity (66.8%), specificity (75.0%) and AUC (0.551). In contrast 71 (69.3%) patients were positive IgG by ELISA with sensitivity (69.8%), specificity (31.2%), and AUC (0.505). Furthermore 60 (58.8%) patients were positive ICT for serum IgG with sensitivity (45.3%), specificity (62.5%), and AUC (0.539). While 48 (62.34%) patients were positive ICT for stool Ag with sensitivity (79.2%), specificity (58%.6) and AUC (0.689).*
Conclusion: *Serological tests are simple, fast and easy technique but had many limitations for screening of H. pylori infection so the serological tests for diagnosis of H. pylori should be confirmed with other more diagnostic tests.*

Key words: *H. pylori* infection, Dyspeptic Patients, ELISA, ICT, PCR, Sudan.

Introduction

H. pylori are Gram negative spiral shaped motile bacteria with polar flagella which are attached at their ends sides giving S-shape [1]. *H. pylori* originally classified in the genus Campylobacter depend on phenotypic and growth patterns features similarity, but RNA sequencing, cellular fatty acid profiles, and other taxonomic characteristics indicate that the micro-organism should be separate in the genus Helicobacter [2]. Globally over 80% of individuals infected with *H. pylori* are asymptomatic [3]. In Africa the prevalence of *H. pylori* was reported as 79.1% most likely due to socioeconomic factors. Transmission of *H. pylori* infection supported mainly with hygienic environment, higher incidences of infection among people clustering, poor sanitation and socioeconomic status [4]. There are many external sources have been transmit *H. pylori* infection like contaminated food, water, feces, saliva and vomit [5]. *H. pylori* remarkable with ability to establish lifelong colonization of gastric human especially of the antrum site. The bacteria are present in large

numbers in the mucus overlying mucosa where the pH is alkaline [6, 7].The gastrointestinal *H. pylori* is commensal to hostile bacterium because of its low partial oxygen pressure and the presence of high concentrations of gastric acid and digestive enzymes [8] .*H. pylori* requires growth supplementation like fetal calf serum (FCS), bovine serum albumin for reduces the toxicity of fatty acids, which inhibit the growth of bacteria, 2-6-dimethyl-β-cyclodextrin (CD), acts in the same way by binding the toxic metabolites produced by the bacteria. Addition of cholesterol and amino acids enhances cell viability. *H. pylori* penetrate the mucus lining of duodenum and esophagus [9]. Untreated *H. pylori* infection causes serious complications like gastric ulcers and peptic ulcer, which are regarding as risk factors for carcinoma of gastric and mucosa association lymphoid tissue (MALT) [10, 11]. Risk of peptic ulcers develops in 10 to 20% lifetime in individuals infected with *H. pylori* and in 1 to 2% risk of acquiring stomach cancer [12]. Inflammation of the corpus is more likely lead to gastric ulcers and gastric carcinoma while inflammation of the pyloric antrum is more likely lead to duodenal ulcers [13]. The immune response to *H. pylori* infection includes both the humoral immunity by production of antibodies (local and systemic) and cell mediated immune response [14]. Cascade of immune system response against *H. pylori* initiate with antibodies production by B lymphocytes. IgM is the first one antibody produced which persist for several days, subsequently develops IgA persist for several weeks and then IgG persist for several months or years. The host could be harmed by the immune response due to direct damage and affect function of epithelial cells, also the immunity can be lead to chronic superficial gastritis. The host epithelial cells which damaged allow *H. pylori* to enter the lamina propria, where direct interaction with innate and acquired immune response cells occurs. T helper-1 and T helper-2 cells are activated and mediate the responses to infection. Many relative balances of these responses are variable among different people and influence the density of the bacteria, severity of the gastritis, chronic superficial gastritis and the cancer risk [15]. The serological approach is non invasive method to screening *H. pylori* infection and useful for detecting recent or past exposure for the pathogen. Traditional serological tests mainly detects the *H. pylori* specific immunoglobulins such as IgA, IgM and IgG against *H. pylori* in serum rather than the saliva and urine due to the lower titer of antibodies in these samples in comparison with serum [16]. Testing of *H. pylori* antigens in feces use specific antibody to identify catalase or flagella proteins. Many studies evaluated the accuracy of stool antigen testing of both initial diagnosis and post treatment follow- up [17]. Other invasive methods utilize biopsy samples are use for diagnosis of *H. pylori* requiring histological examination, culture, rapid urease test, molecular applications and fluorescent in-situ hybridization [18].

Materials and Methods

The study is descriptive cross-sectional study carried out to diagnose the *H. pylori* infection among 102 adult patients from both sex (age range between 20–70 years) attending Gezira Center for G.I.T Endoscopy and Laparoscopic Surgery during period between 2016 – 2019. According to the clinical data these patients clinically suspected to be infected with *H. pylori*. Ethical approval of study was taken from Ministry of Health, Gezira State; while informal consent was taken from each participant. All procedures of diagnosis were performed in Molecular Biology and Advanced Research Laboratory, Faculty of Medical Laboratory Sciences, University of Gezira. Antrum biopsy sample was collected by gastroenterologist using (Fujunon Model) endoscope under standard techniques. Three mls of blood sample and one spoon full of stool sample were collected. ELISA techniques for detection of serum IgG and IgA antibodies against *H. pylori* antigen were performed using EURO IMMNNE ELISA Kits, PerkinElmer Company–Germany.

Immunochromatography test (ICT) to *H. pylori* fecal antigen and detect serum IgG antibody against *H. pylori* antibody and serum IgG antibody against *H. pylori* antigen using were tested using with Hangzhou All Test Biotech Company–China.

DNA extraction from biopsy sample was performed using Jena AG, innPREP AND mini kit. Extracted DNA was stored at -20^{0}C until used.

Specific primer of 16s rRNA gene with length 502 bp (forward sequence: "GCGCAATCAGCGTCAGGTAATG" and Reverse sequence: "GCTAAGAGAGCAGCCTATGTCC (Macrogen Company, South Korea).

Extracted DNA was amplified by PCR using 5µl of 16s rRNA primer, 4µl master mix containing (Tag DNA polymerase, PCR buffer, dNTPs and $MgCl_2$), 1µl of BSA 22%, 0.5 µl $MgCl_2$ and 3.5 µl D.W to complete the volume 20 µl, then 35 cycles in thermal cyclers was held for amplification with an initial denaturation (92 ^{0}C for 10 min), denaturation (95 ^{0}C for 30 sec), annealing of primer (60 ^{0}C for 1min), extension (72 ^{0}C for 40

sec) with final extension (72 ^{0}C for 5min). PCR products examined in gel electrophoresis with positive control of *H. pylori* DNA and molecular size marker 100-bp DNA ladder (Boehringer Mannheim, Germany). DNA was visualized under 0.96 intensity ultra violet (UV) light using gel documentation system (Model: OMNIDOC) as show in figure 1.

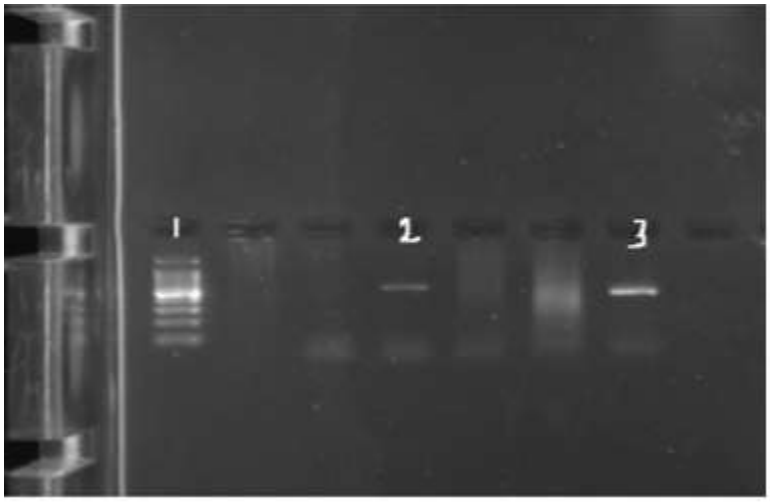

Figure 1. PCR amplification of *H. pylori* 16s rRNA gene with 502 bp on 1.5 agarose gel electrophoresis. **Lane 1** ladder: MW 100-1000 bp. **Lane 2** control positive, **Lane 3** positive samples.

Results

Study was conducted on 102 patients (45% male, 55% female), their ages ranged between 20 – 70 years (mean 46.1 ± 13.1 years) (Table 1). The epigastric pain is most common clinical finding among patients (Table 2). The frequency of *H. pylori* infection in biopsy samples by detection of 16s rRNA gene using PCR methods was 53 (51.9 %) (Table 3). Frequency of 16s rRNA gene using PCR methods among patients had previous *H. pylori* infection was 46.2% compared with 38.2% of patients with recent H. pylori infection (*P value* = 0.347) (Table 4). The Sensitivity, Specificity and AUC for ELISA IgA were (66.8, 75 and 0.55 respectively); for ELISA IgG were (69.8, 31.2 and 0.51 respectively); for ICT stool Ag were (79.2, 58.6 and 0.68 respectively); and for ICT IgG were (45.3, 62.5 and 0.53 respectively) (Table 6).

Table 1. Demographic data of study group.

Gender	No.	Age groups	Mean (years)	STD (years)
Male	49 (45%)	20 – 40 (22%) 41 – 55 (32%) 56 – 70 (46%)	46.1	13.1
Female	53 (55%)			

Table 2. Clinical finding among study group.

Symptom	Frequency		Odd.	C. l (95%)	
	Pos.	Neg.		Lower	Upper
Epigastric pain	71 (69.6%)	31 (30.4%)	0.357	0.133	0.961
Vomiting	30 (29.4%)	72 (70.6%)	1.778	0.696	4.543
Dyspepsia	28 (27.5%)	74 (72.5%)	1.500	0.612	3.678
Blenching	15 (14.7%)	87 (85.3%)	1.451	0.438	4.811
Diarrhea	14 (13.7%)	88 (86.3%)	1.911	0.449	8.127

Table 3. Frequency and percentage of positive and negative *H. pylori* (16s rRNA gene) in biopsy samples using PCR among study group.

Valid		Frequency	Percent %
	Positive	53	51.9

16s rRNA gene by PCR	**Negative**	49	48.1
	Total	102	100.0

Table 4. association between detection of 16s rRNA gene in biopsy samples using PCR among and previous *H. pylori* infection.

History		**PCR**		**Total**	**P. value**
		Positive	**Negative**		
Previous infection	**Yes**	12 (46.2%)	14 (53.8%)	26 (32.5%)	0.347
	No	29 (38.2%)	47 (61.8%)	76 (67.5%)	

Table 5. Frequency and percentage of positive and negative *H. pylori* using ELISA IgG, ICT stool Ag, ICT IgG and ELISA IgA.

Frequency/Percentage			
Valid	**Positive**	**Negative**	**Total**
ELISA IgG	71 (69.6%)	31 (30.4%)	102
ICT Stool Ag	48 (62.3%)	29 (37.7%)	77
ICT IgG	60 (58.8%)	42 (41.2%)	102
ELISA IgA	54 (52.9%)	48 (47.1%)	102

Table 6. Sensitivity, Specificity and AUC for ELISA IgA, ELISA IgG, ICT stool Ag and ICT IgG.

Methods		**PCR**		**Total**	**Sensitivity**	**Specificity**	**AUC**
		Positive	**Negative**				
ELISA IgA	**Positive**	19	21	31	66.8	75.0	0.55
	Negative	35	36	71			
ELISA IgG	**Positive**	38	33	71	69.8	31.2	0.51
	Negative	16	15	31			
ICT Ag	**Positive**	38	12	50	79.2	58.6	0.68
	Negative	10	17	27			
ICT IgG	**Positive**	30	29	59	45.3	62.5	0.53
	Negative	24	18	42			

Discussion:

H. pylori infection is regard as critical health problem worldwide, especially in the developing countries when the prevalence approximately estimated as 90%. Significant differences in prevalence across the world; which relies particularly on socioeconomic conditions, overcrowding, poor sanitation, hygiene and behavior traits of the patients [19]. *H. pylori* is inhabits the stomach of > 50% of humans and has been established as a major etiological factor in pathogenesis of chronic gastritis, gastric atrophy, peptic ulcer diseases, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma [20].

The current study used PCR technique to evaluate serological investigations (ELISA and ICT) for *H. pylori* infection in 102 dyspeptic patients in Gezira State, Sudan during 2016 -2019.

According to demographic data this study included 45% male and 55% female (55%). The frequency of *H. pylori* among study group was 51.9% using 16s rRNA gene detection by PCR, referring to study findings on equitation of clinical presentation, epigasteric pain 71 (74%) and vomiting 30 (25.8%) had high common frequency. This results agree with findings by Srinivas *et al.*, that reported the epigastric pain (28%) and vomiting (23.2%) [21]. In this study serum antibody (IgG) tested by ICT obtained less sensitivity (45.3%) and specificity (62.5%), furthermore serum antibody using ELISA IgG showed sensitivity (69.8%) and specificity (31.2%). These findings are lower than that reported by Rahman *et al*., They evaluated IgG test by (ICT /ELISA) in a total of 82 Bangladesh patients. Their results showed ICT as sensitivity 90.1% and specificity 80.9%, while ELISA showed sensitivity 96.7% and specificity 42.8% [22], these findings may be due to the different in immunological status and geographical area of population. In a similar study done in Iran for IgG detection using ELISA, the results showed the sensitivity was (91.3%) and specificity (55.6%) [23]. The study disagree with Hassan *et al*., evaluated 104 patients used urease and histology as the gold standard obtained IgG level had a sensitivity of 58.6%, specificity of 61.3% [24], this may be due to differences in the gold standard of these studies. The high seropositivity of IgG may be due to previous infection with *H. pylori* when tested by PCR. Serum ELISA IgA in the presented study showed sensitivity (66.8%), specificity

(75.0%). This finding agree with results in the same study in Iran that showed ELISA IgA had sensitivity 64% and specificity 85% [24]. Generally variation in serum Abs detection between studies could be due to differences in prevalence of *H. pylori* between populations, various in geographic locations and ethnic population which affect with variety in socioeconomic status, health status, crowding and other factors. All these reasons lead to differences in prevalence which have an obvious impact on statistical indexes.

Stool antigen test (SAT) was report in current study had high sensitivity (79.2%) but low specificity (58.6%). This finding agree with study done by Ceponis *et al.*, in Brazil which studied SAT among 122 patients and showed high sensitive (92.45%) but low specific (81.25%) [25]. On the other hand disagree with Khadka *et al*., study that showed the sensitivity was 53.8% and specificity was 88.2% [1]. This variations justified according to, that SATs are hold different limitations like sample homogenize if unformed or watery, when *H. pylori* specific antigens in this case is more diluted. Temperature, storage time and the interval between stool sample collection and measurement also affect the results of SAT. The choice of test kit depends on the sensitivity and specificity in each region and the circumstances of each patient.

Conclusion

Serological tests had more limitations for screening of *H. pylori* infection. Geographical variations, immunological patterns, hygienic habitats and socioeconomic status may play major role in prevalence of infection and this is affecting the evaluation of serological tests. There for sero-detection of *H. pylori* need more confirmation with other confirmative diagnostic tests.

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Competing interests:

The authors declare that they have no competing interests.

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