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Molecular Detection of *H. Pylori* from Gastric Biopsies of Dyspeptic Patients Attending Endoscopy Center, Gezira State, Sudan

YOSRIA MOHAMMED ELSIDDIG
ALAA FAROGE IBRAHIM
Lecturer, Department of Medical Microbiology
Faculty of Medical laboratory Sciences, University of Gezira Sudan
ROA OSMAN ELSUMANI KOKO
Department of Molecular Biology
Faculty of Medical laboratory Sciences, University of Gezira, Sudan
OMER H. M. ARABI
Professor, Department of Basic Science
Faculty of Animal Production, University of Gezira, Sudan
MOHAMED IBRAHIM M. MALIK
Associated Professor, Department of Medicine
Faculty of Medicine, University of Gezira, Sudan
ADAM DAWOUD ABAKAR
Department of Medical Parasitology
Faculty of Medical laboratory Sciences, University of Gezira, Sudan
KHALID ABDELSAMEA MOHAMEDAHMED[1]
Lecturer, Department of Hematology and Immunology
Faculty of Medical laboratory Sciences, University of Gezira, Sudan

Abstract

Background: *H. pylori are Gram negative bacteria cause most common of gastrointestinal tract infections worldwide. H. pylori can lead to serious symptomic or a symptomic illness including ulcers, gastritis, doudenitis, oesophagitis. Serious complications like gastric atrophic, mucosa-associated lymphoid tissue (MALT) lymphoma can occur. H. pylori posses cag A gene which is a virulent factor and marker for the pathogenic strain. This strain associated with greater*

[1] Corresponding author:khalid.abdelsamea@hotmail.com

inflammations and increased the risk of developing both peptic ulcer diseases and gastric carcinoma.

Objective: *The study conducted to focus on detection of H. pylori and cag A gene using polymerase chain reaction (PCR) method in biopsy samples from upper gastrointestinal diseases patients in Gezira State, Sudan.*

Materials and Methods: *Descriptive cross - sectional study was carried out during 2016 – 2019 in Gezira State, Sudan. A total of 102 antrum biopsy samples were collected from adult male and female, their age between (20 – 70 years). Biopsy sample collected by gastroenterologists at Gezira Center for G.I.T. Endoscopy and Laparoscopic Surgery. H. pylori DNA extracted to apply the PCR technique in order to investigate H. pylori infection and cag A gene. In this study PCR for 16s rRNA accepted as gold standard method to identify the H .pylori.*

Results*: In 102 adult dyspeptic patients (45% male, 55% female, mean of age 46.1 ± 13 years). H. pylori was detected in 53 (51.9%) biopsy samples using PCR 16s rRNA, 22 (41.5%) were positive for cag A. Epigastric pain was a common clinical feature in individuals infected with H. pylori 71 (74%), dyspepsia 28 (29.5%) and vomiting 30 (25.8%). H. pylori infection predominant in gastritis and ulcer patients. Frequency of H. pylori positive cag A was common among in ulcers, gastritis, duodenitis and esophagitis patients. Out of 102 patients 9% reported mass endoscopy finding from them 56% infected with H. pylori when tested by the PCR in those positive H. pylori 80% reported positive cag A.*

Conclusion: *Frequency of H. pylori infection using 16s rRNA is 53 (51.9%) predominant in ulcers and gastritis patients. Cag A gene highest in gastritis and ulcers patients compare with other diseases, this gene play a role for determination the clinical outcome of H. pylori infection.*

Keywords: *H.* pylori, 16s rRNA, *Cag A*, antrum biopsy, PCR.

Introduction

Helicobacter pylori is Gram negative bacilli, motile and non-spore-forming [1]. It grows well between pH 6 and 8 in artificial culture

media. It is capable to colonize acidic environment of human stomach because it has evolved a variety of acid resistant mechanisms [2]. Diseases associated with this bacterium were highly prevalent during the 19th century, it is responsible for most stomach and duodenal ulcers and many cases of stomach inflammations (chronic gastritis) [3]. The prevalence of *H. pylor*i infection varies widely by geographic area, age, race, socioeconomic status ethnicity, household crowding, and migration from high prevalence regions, interfamilial infection and genetic predisposition [4]. In developing countries, the prevalence of *H. pylori* infection is 80%. *H. pylori* transmitted with oral rout through contaminate food and water [5]. Infection with *H. pylori* associated with peptic ulcer, gastritis, gastric atrophic, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric carcinoma [6].

The *H. pylori* bacterium hold the 16s rRNA gene which is one of the specific targets to confirm *H. pylori* infection and positive amplification of *H. pylori* DNA considered as a direct evidence of the presence of the pathogen. 16s rRNA implicated in the binding of transfer RNA to messenger RNA (mRNA), it used for phylogenetic studies as it is highly conserved gene due to the slow rates of evolution [7].

Cag A protein is one of several factors associated with *H. pylori* infection; it is present in approximately 60 – 70% of *H. pylori* clinical strains [8]. This gene is marker for the pathogenic *H. pylori* and has type IV secretory pathway. This pathway encodes a secretion system involved in the produce of virulence determinants and influences the secretion of IL-8, involved in the severity of the disease, also *cag A* increase the risk of developing peptic ulcer disease and gastric carcinoma. The protein subsequently affects host cell gene expression, inducing cytokine release and altering cell structure [9]. *Cag A* is characterizing with the diversity because *H. pylori* pathogen and their hosts both co-evolve due to the migration through the world; these recurrent migrations provided the potential for genetic changes of both pathogen and hosts [10].

Invasive methods like urease test, histology test, culturing technique, PCR and non invasive methods like serological tests and urea breath test are use for detection of *H. pylori* in clinical samples.

The *cag A* protein detect by ELISA in blood and PCR test in biopsy sample [11].

Polymerase chain reaction (PCR) has been successfully method used to detect the *H. pylori* in biopsy sample because it highly sensitive and specific [12].

Materials and Methods

This was descriptive cross sectional study carried out between 2016 – 2019 in Gezira State, Sudan. Population of study included both adult male and female patients aged between (20 – 70 years). They referred for upper gastrointestinal endoscopy and clinically suspected to *H. pylori* infection. The patients had not received antimicrobial agents in the two weeks prior to examination. Antrum biopsy was collected under standard techniques by physician using (Fujunon Model) endoscope at Gezira Center for G.I.T. Endoscopy and Laparoscopic Surgery. Ethical approval of study was taken from Ministry of Health, Gezira State; informed consent was taken from each participants. The samples transported in Eppendorf tube immediately to the laboratory and preserved in - 20 ^{0}C. DNA extraction was performed according to instructions of the manufacturing company analytic Jena AG (innPREP AND mini kit) protocol, then extracted DNA stored at -20 ^{0}C until used. PCR system 9700 (Singapore) used to amplify 16s rRNA and *cag A* genes. Specific primers of 16s rRNA and *cag A* manufactured at Macrogen South Korea Company, with sequence as showed in table 1. All procedures of diagnosis were applied in Laboratory of Molecular Biology, Faculty of Medical Laboratory Sciences, University of Gezira.

Table 1. Sequence of oligonucleotide primers and PCR products size.

Primer	Direction	Sequence 5´- 3´	T_m (°C)	Product size
16s rRNA	Forward	GCGCAATCAGCGTCAGGTAATG	62	502 bp
	Reverse	GCTAAGAGAGCAGCCTATGTCC		
Cag A	Forward	AATACACCAACGCCTCCAAG	53	400 bp
	Reverse	TTGTTGGCGCTTGCTCTC		

Extracted DNA was amplified by PCR to detect the *H. pylori* using 0.5µl of 16s rRNA primer, 4µl master mix containing (Tag DNA polymerase, PCR buffer, dNTPs and $MgCl_2$), 1µl enhancers of BSA

22%, 0.5 µl $MgCl_2$ and 3.5 µl D.W to complete the volume 20 µl. The amplification was held by 35 cycles in thermal cyclers, each cycle has three steps. 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).

Cag A gene was detected using amplification reaction as the same protocol of 16s rRNA amplification except the primer was added in amount 10 µl. The reaction carried out with 35 cycles on thermo cycler under an initial denaturation (92 ^{0}C for 10 min), denaturation (95 ^{0}C for 30 sec), annealing of primer (53 ^{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 parallel with positive controls and molecular size marker 100-bp DNA ladder (Boehringer Mannheim, Germany).

Upon electrophoresis, DNA was visualized under 0.96 intensity ultra violet (UV) light using gel documentation system (Model: OMNIDOC) show figure 1 and figure 2.

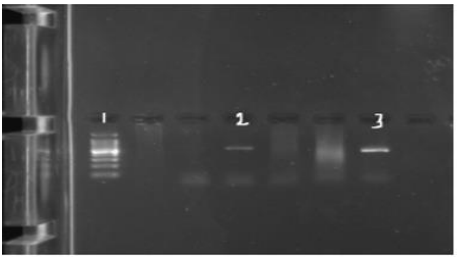

Figure 1. PCR amplification of *H. pylori* 16s rRNA gene with 502 bp on 1.5 agarose gel electrophoresis. Lane 1 ladder: MW 100-1000 bp. Lane 2 control positive, Lane 3 positive samples.

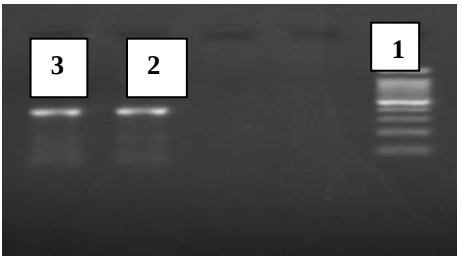

Figure 2. PCR amplification of *H. pylori* positive *cag A* gene with 400 bp on 1.5 agarose gel electrophoresis. Lane 1 ladder: MW 100-1000 bp. Lane 2 control positive, Lane 3 positive samples.

Results

H .pylori tested in 102 patients, male (45%) and female (55%) and mean of age was 46.1 ± 13.1 years (Table 2). Frequency of *H. pylori* infection was 51.9 % (53) yield positive for 16s rRNA (Table 3). 22 biopsy samples (41.5%) were positive for *cag A* gene (Table 4). The frequency of prevailing symptoms were epigastric pain71 (74%), dyspepsia 28 (29.5%) and vomiting 30 (25.8%) are common clinical features among the study group (Table 5). Out of 102 patients, 9 reported mass endoscopy. When subjected to PCR screening, 5 out of 9 diagnosed as positive for *H. pylori* (56%). Further screening of 5 positive mass *H. pylori* show that 80% (4/5) reported *cag A* positive. Endoscopy findings of the study group were normal, gastritis, oesophagitis, mass, duodenaitis and ulcer. Strong correlation was observed between *H. pylori* positivity, and certain symptoms such as epigastric pain (74%), dyspepsia (29.5%) and vomiting (25.8%). Moreover, 9.4% (4/53) that have been diagnosed as positive for 16s rRNA did not show any clinical symptoms indicative for *H. pylori* infection (Table 6). A 20 (37.7%) male and 12 (22.6%) were positive samples for *H. pylori* infection detected with PCR 16s rRNA and in the same time were positive *cag A* gene (Table 7).

Table 2. Demographic data of study group.

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

Table 3. Frequency and percentage of positive and negative biopsy samples for 16s rRNA *H. pylori* using PCR.

Valid		Frequency	Percent %
PCR for 16s rRNA *H. pylori*	Positive	53	51.9
	Negative	49	48.1
	Total	102	100.0

Table 4. Frequency and percentage of positive and negative of *Cag A* gene in biopsy samples using PCR.

Valid		Frequency	Percent %
PCR for *Cag A* gene	Positive	22	41.5
	Negative	31	58.5
	Total	53	100.0

Table 5. Clinical features of upper GIT diseases among study group.

Symptom	Frequency	Odd.	C. l (95%)	
			Lower	Upper
Epigastric pain	71 (74.0%)	0.357	0.133	0.961
Dyspepsia	28 (29.5%)	1.500	0.612	3.678
Vomiting	30 (25.8%)	1.778	0.696	4.543
Blenching	15 (13.7%)	1.451	0.438	4.811
Diarrhea	14 (9.3%)	1.911	0.449	8.127

Table 6. Association between PCR 16s rRNA in biopsies and common clinical symptoms.

Symptom	PCR of *H. pylori* 16s rRNA		Total
	Positive	Negative	
Epigastric Pain*	42 (79%)	11 (21%)	53 (100%)
Dyspepsia	18 (34%)	35 (66%)	53 (100%)
Vomiting	17 (32.1%)	36 (68%)	53 (100%)

Table 7. Association between positive, negative 16s rRNA versus *cag A* gene among the different gender.

16s rRNA / *Cag A*			
	Male	Female	Total
Positive	20 (37.7%)	12 (22.6%)	32 (60.37%)
Negative	8 (15%)	13 (24.5%)	21 (39.62)

Discussion

The study investigated 102 antrum biopsy samples for *H. pylori* and virulent factor *cag A* using polymerase chain reaction technique after endoscopy for symptomatic upper GIT patient. In this study 16s rRNA used as gold standard for *H. pylori* detection. 45% were male and 55% female. Frequency of *H. pylori* in biopsy samples was 53 (51.9%), these result was compared with finding by Khadka *et al.,* in 2018 in 90 patients found 24 cases were positive for *H. pylori* when using PCR

technique [13], also Srinivas *et al.,* study in 100 gastric biopsy samples and showed (36%) were positive for *H. pylori* in dyspeptic patients [5]. These results indicate to possible associated between upper GIT diseases and *H. pylori* infection. A symptomatic *H. pylori* infection in this study discussed may due to long period of time for bacteria colonization in human stomach until it causes severe infection or due to recent infection. Endoscopy findings analyzed by chi square test cross tabulation method yield (*P value* = 0.041), where that resulted by Mona *et al*., in 2017 in Sudan was (*P value* = 0.036) [14]. In this study 22 (41.5%) samples were positive for *cag A* using PCR. This result compared with study done by Peters *et al*., in 2001 included sixty two gastric biopsy screened by PCR assay for the presence of *cagA* in UK patients, forty one were obtained positive result [15]. In conducted study, *cag A* was reported in ulcer (72%) and (68%) in gastritis patients compared with study done by Bindayna *et al*., in 2006 conducted to determine the prevalence of *cag A* among 100 dyspeptic patients in Bahrain using PCR with specific primer hold 400 bp, they detected *cag A* in 59 (59%) in biopsy samples also they found *H. pylori* positive *cag A* was significantly higher in ulcer patients (80%) and gastritis patients (47%) [16]. The different prevalence results justify according to varies in immunological status and geographic regions of studied population.

Presented study found positive association between mass endoscopy finding and *cag A* gene presence, that indicated to pathogenic *H. pylori* and may lead to malignancy equation. *H. pylori* positive *cag A* in this study was predominant in gastritis and ulcer patients. According of literature review *H. pylori* is recognized as important agent of gastritis, the main risk factor for peptic ulcer disease and gastric carcinoma [17, 18].

Conclusion

This study found *H. pylori* infection associated with ulcers and gastritis in upper GIT patients and concluded that c*ag A* gene typing by PCR importance to differentiating between pathogenic and nonpathogenic strains. The positive *H. pylori cag A* gene associated with greater inflammation and increased risk of ulcers and cancer.

PCR for detection of *cag A* gene is important and usefulness as an integral part in diagnosis of *H. pylori* infections.

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