Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 1 January 2023

Research Article

Molecular Detection of *Helicobacter pylori* and its *CagA* Gene in Upper Gastrointestinal Disease Suspected Patients Living in Shendi Locality, Sudan

Amel Abd Elhafeez S Ali[1]*, Hadia Abass Eltaib[1], Ghanem Mohammed Mahjaf[1], Mazin Babekir Musa Bashir[1] and Babbiker Mohammed Taher Gorish[2]

[1]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Sudan*

[2]*Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Sudan*

***Corresponding Author:** Amel Abd Elhafeez S Ali, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Shendi University, Sudan.

Received: November 15, 2022

Published: December 29, 2022

© All rights are reserved by **Amel Abd Elhafeez S Ali., *et al.***

Abstract

Over half of the world's population are chronically infected with *Helicobacter pylori (H. pylori),* the only bacterium that the WHO has classified as a carcinogen due to its connection to the emergence of gastric cancer. The purpose of this descriptive study was to determine the frequency of *H. pylori* in a patient suspected with upper gastrointestinal (UGIT) disease using ICT and nested PCR as well as detection of the *CagA* gene in the Shendi locality. A total of 100 stool samples were collected from patients with UGIT symptoms (60% males and 40% females) with a mean age of 37.2 11.8. Samples were analyzed for the presence of *H. pylori* antigen by using rapid ICT test , while nested PCR was used to detect *H. pylori* and its associated *CagA* gene. Data was collected using a structured questionnaire, and the results were analyzed using (SPSS version 22). Our findings show that the frequency of *H. pylori* among patients is 89% by ICT and 65% by PCR, respectively. However, the frequency of *CagA* gene among positive PCR patients was 58.5%. We conclude that there was a high prevalence of *H. pylori* infection with a high *CagA* gene producing strain among Sudanese patients in the Shendi locality.

Keywords: *H. pylori*; *CagA* Gene; ICT; Nested PCR; Stool Sample; Sudan

Abbreviations

PCR: Polymerase Chain Reaction; ICT: Immunochromatogaphy Test; *H. pylori*: *Helicobacter pylori*; ELISA: Enzyme Linked Immunosorbant Assay; UBT: Urea Breath Test; SAT: Stool Antigen Test

Introduction

Helicobacter pylori infection has been linked to several diseases, including simple and complicated ulcers, mucosa-associated lymphatic tissue lymphoma, peptic ulcer disease, and gastric cancer. Continuous collaborative effort among clinical staff, pathologists, and biotechnologists is required for proper management of these harsh circumstances [1-3].

H. pylori infection increases a human lifetime chance of developing stomach cancer and peptic ulcers by 1% to 2%. Inflammation of the pyloric antrum causes duodenal ulcers, whereas inflammatory response of the corpus (body of the stomach) causes gastric ulcers and stomach carcinoma [4-6]. The organism is also thought to be the root cause of other human illnesses such as hematologic and autoimmune disorders, diabetes mellitus, and metabolic disorders.

Although infections with *H. pylori* affects nearly 50% of people around the world, rates of prevalence, occurrence, age distribution, and infection sequelae vary markedly between developed and developing nations. The prevalence of *H. pylori* infection is declining in developed countries, but it remains high in developing nations [7]. In Sudan, the infection rate is estimated to be 80% [8].

Because the stomach has a limited population of bacteria, the sensitivity of the direct test decreases. As a result, a variety of indirect tests for infection with *H. pylori* have been developed, including antibody-based tests such as serological testing, urine samples, urea breath testing (UBT), as well as antigen detection testing (SAT) [9]. Regardless of antibiotic resistance, using bismuth-based quadruple treatment appears as the most effective way to treat *H. pylori* activity and almost entirely maintains high eradication rates [10,11]. The purpose of investigating the *H. pylori* genome is to gain a better understanding of the organism's pathogenesis and disease-causing potential. Approximately 29% of the gene loci have a colonial expansion defect when mutated. The Cag pathogenicity island, a common gene sequence thought to be responsible for pathogenesis, is around 40 kb long and includes more than 40 genes in two sequenced strains. *H. pylori* strains isolated from infected but asymptomatic humans usually have limited this pathogenicity island [4].

The *cagA* gene encodes one of the primary virulence proteins produced by *H. pylori*. Bacterial strains carrying the *cagA* gene have ulcer-causing potential. The *cagA* gene produces a protein with a long sequence of amino acids (1186). The cag pathogenicity island (PAI) is made up of about 30 genes, some of which encode for the complex type IV secretion system. Based on the low GC-content of the cag PAI in contrast to the remainder of the Helicobacter genome, the island was most likely acquired through horizontal transmission from another species of bacteria [4,12]. In Western countries, approximately 50-70% of *H. pylori* strains hold the cag pathogenicity island genes, which might also increase *H. pylori* pathogenicity [13]. After *H. pylori* has attached to the stomach epithelial cells, the type IV secretion system demonstrated by the cag PAI "injects" the peptidoglycan, an inflammatory agent, into the cells. The injected peptidoglycan is recognized by the immune sensor Nod1 in the cytoplasm, which causes the appearance of cytokines that trigger inflammation [14].

Data about *H. pylori* in Shendi locality is appeared to be few and most of the previous study were done to detect just the presence of the infection evidence by using *H. pylori* sero diagnosis technique. Therefore here our study was aimed to detect the presence of infection as well as *CagA* gene in stool samples of Sudanese patients living in Shendi Locality.

Materials and Methods

Design of setting

This is a descriptive cross-sectional study that was carried out between January 2018 and April 2019. The research was carried out in Shendi, Sudan's River Nile state, about 173 kilometers north of Khartoum's capital. Shendi is located on the east bank of the Nile and covers an area of 17 kilometers square. Patients with upper gastrointestinal symptoms were included in the study. A total of (100) samples were chosen based on available facility and cost.

Stool sampling

A stool sample was collected using a sterile stool container, about 2 g of stool was put in an Eppendorf tube and frozen in (-20c) for DNA extraction, the remaining sample was tested by using ICT stool test.

Data collection tools and analysis

Data was collected using a self-administrated pre-coded questionnaire which was specifically designed to obtain information that helped in the study. The collected data code in the master sheet and proceed for analysis using SPSS version [20].

H. pylori antigen detection by ICT method

The *H. pylori* antigen rapid test is a double antibody-sandwich technique-based lateral flow chromatographic immunoassay. The test cassette is made up of two parts: (1) a burgundy-colored conjugate pad with *H. pylori* antibodies conjugated with color particles, and (2) a nitrocellulose membrane strip with a test band (T band) and control band (C band). The T band is pre-coated with non-conjugated *H. pylori* antibodies, and when a sufficient amount of the test specimen is dispensed into the cassette's sample well, the specimen migrates If *H. pylori* antigen is present, it will bind to *H. pylori* antibodies conjugates. The pre-coated *H. pylori* antibodies then capture the immunocomplex on the membrane, resulting in the formation of a burgundy-colored T band that represents a positive

test result for the *H. pylori* antigen. The sample was obtained, the test device was removed from the foil, carefully broken off the tip, and 2 drops of the sample were squeezed into the sample well of the cassette. The result was ready after 10 minutes, and a positive result was confirmed by the presence of red line wear, while a negative result was indicated by the absence of red line.

DNA extraction from stool samples

Stool sample was placed in a 2 ml micro centrifuge tube and DNA extraction was done by hand using QIAamp Fast DNA Stool Mini Kit (Qiagen) with a final elution volume of 200 L, as directed by the manufacturer. All extracted DNA samples were stored at 20°C until molecular analysis. DNA concentrations were not assessed prior to the PCR runs, as they are not under diagnostic standard circumstances according to our methods [15].

PCR for detection of *H. pylori*

A procedure for performing PCR, which was based on the DNA sequence of a species-specific protein antigen, was followed. Following the procedure, a semi-nested PCR was created in the lab using the upstream primer (5′-TGGCGTGTCTATTGACAGCGAGC-3′) and the nested primer (5′-TGATCACTGCATGTCTTACTTTCATGTTTTT-3′) from the main PCR. These oligonucleotides correspond to the reported sequence residues 474 to 496 and 682 to 652, respectively. For thermal cycling, reactions were carried out in a volume of 50 l using a TRIO-Thermo block. . In a typical PCR incubation buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.001% gelatin), 0.5 l of primary PCR product was added to a reaction mixture containing the four deoxynucleotides at 100 M each, 0.1 M each primer, and 0.5 U of the Tag DNA polymerase. The semi-nested PCR was performed with an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 68°C for 1 minute, and extension at 72°C for 45 seconds. After the last cycle, the mixture was incubated for 5 minutes at 72°C. The amplification result was electrophoresed on a 1.5% agarose gel using a normal procedure, and a band at 209 bp was detected and considered a positive PCR result [15].

PCR amplification using the *CagA* primer

PCR was performed with the primer sets cagA-F (5′-CAA TGA ATT TTT GAT CCG GG-3′) and cagA-R (5′-GAT AAC AGG CAA GCT TTT GAG GGA3′). The 25 l reaction mixture contained 1x PCR buffer, 1.5Mm magnesium chloride, 200M of each d NTP, 20 pmol of each primer, and 1U Tag DNA polymerase. An initial denaturation at 94°C for 5 minutes was followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute, followed by a final extension at 72°C for 5 minutes. A 5 µl PCR product was separated on 1.5% Agarose gel using TBE buffer (Tris Boric Acid), and a 100 bp ladder was employed as a DNA molecular weight standard and stained with 0.15% Ethidium bromide, then the result was observed using a UV gel documentation system, with the PCR product size 349 bp (17).

Results

A total of (100) stool samples were collected from patients suspected of upper gasterintersinal diseases, 60(60%) were males while 40(40%) were females. Regarding the distribution of age within gender, the patient was categorized into two groups, the first one from (15-40 years) 23 were male while 15 were female and the other group from (41-65 years) 37 of them were male and 25 were female (Table 1). Regarding the patient's symptoms, 29 presented with abdominal pain, 25 with vomiting, 22 with heart pain, 18 with nausea, and 6 patients have combined symptoms. The frequency of *H. pylori* infection among the study population detected by ICT and nested PCR techniques were 89% and 65% respectively (Table 2) (Figure 1). On the other hand, the frequency of *the CagA* gene among infected patients was 58.5% using PCR (Figure 2). Our results demonstrated that gender have no effect on the result of ICT test as well as PCR analysis for detection of *H. pylori* with P-value of 0.794 and 0.466 respectively (Table 3). However, age group 41-65 years are more likely to be infected with *H. pylori* by using ICT test, while PCR analysis determined more *H. pylori* infection among age group 15-40 (Table 4).

Age group	Gender		Total
	Male	Female	
15 - 40	23	15	38
41 - 65	37	25	62
Total	60	40	100

Table 1: Distribution of gender according to age group.

Techniques	Positive	Negative	Total	Percentage (%)
ICT	89	11	100	100
PCR	65	35	100	100

Table 2: Frequency of *H. Pylori* infection among study population using (ICT&PCR) techniques.

Figure 1: Detection of H. pylori DNA in stool samples by semi nested PCR and analysis of the amplification product (209 bp) by agarose gel electrophoresis and ethidium bromide staining. Lane1 (Negative control) Lane 2 (Positive control). Lanes (4, 5 and 8) positive samples. Lanes (6, 7, 9 and 10) negative samples, L (ladder marker 100 bp).

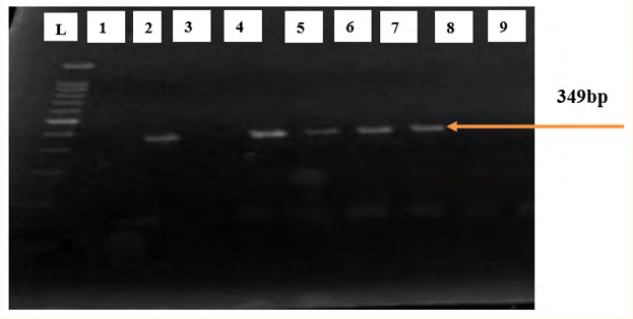

Figure 2: Detection of *CagA* gene in stool samples by PCR and analysis of the amplification product (349 bp) in agarose gel electrophoresis and ethidium bromide staining lines (4-7) infected patients (2) positive control, (3) negative control and (1) 100 bp ladder marker.

Gender	ICT test		PCR for *H. pylori*	
	Positive	Negative	Positive	Negative
Male	53	07	22	22
Female	36	04	43	13
P. value	0.794		0.466	

Table 3: Relationship between age groups and presence of *H. Pylori* infection.

Age group	ICT test		PCR for *H. Pylori*	
	Positive	Negative	Positive	Negative
15-40	31	07	38	16
41-65	58	04	27	19
P. value	0.027		0.001	

Table 4: Correlation between ages and *H. Pylori* infection.

Discussion

H. pylori is one of the most frequent chronic infections in people and is found all over the world. *H. pylori* colonizes more than half of the world's population [18]. The incidence of *H. pylori* infection varies greatly depending on geographical location, patient age, and socioeconomic position, with infection rates ranging from 70-90% in underdeveloped nations to 25-50% in wealthy ones [19]. *H. pylori* is one of the, yet there is no solid information on this illness in Shendi. The purpose of this study was to determine the prevalence of *H. pylori* in 100 patients. The presence of *CagA* gene, the most family virulent gene for *H. pylori* pathogenicity, was detected in 60 (60%) men and 40 (40%) females attending an outpatient clinic in Shendi, Sudan, suspected with upper gastrointestinal disorders. The prevalence of *H. pylori* in patients was found to be 89% by ICT and 65% by PCR, with PCR serving as the gold standard. In this study, the rate of *H. pylori* infection was over 80% (using ELISA) in eastern Sudan [8]. This discrepancy may be attributed to the procedures utilized, and it was substantial when compared to the United States of America and Australia, both of which had a low incidence of *H. pylori* (35.6% and 24.6%, respectively) [20]. The low frequency in the United States and Australia is explained by rising living standards. Variation might be related to sample size [21]. This demonstrates that a PCR test with nested primers is a more sensitive and specific approach for identifying *H. pylori* DNA in stool samples than ICT, and it is considered as a gold standard for diagnosing infection and even for treatment follow-up [22]. Our

study found a very statistically significant link between the presence of infection and age, with a P. value of 0.001. (0.001). There is, however, no statistically significant gender P. value. Although there are several tests for *H. pylori* identification, PCR is more sensitive than other procedures [22]. Also, PCR was used to detect the *CagA* gene, which was found in 38 (38%) of the sample population, which accords with the study done by the University Health Sciences Center [23]. A research at Delft Diagnostic Laboratory found gene detection in a severe instance of *H. pylori* infection [25]. And concurs with Udine University [26]. This explains why (38) of the positive samples were severe. *H. pylori* infection affects 56.3% of Sudanese children, with males outnumbering females [27]. *CagA* prevalence is highest in the United States (85%), followed by Nigeria (93%), and India (96%). In this study, the frequency of samples between (15-40 years is the optimal period to incidence of the infection) represented 66(66%), while other samples between 41-65 years represented 34(34%) [24]. Regarding the age range of the research population, the findings correspond with those of the University Hospital of Kiel [28]. In addition, Tabriz University of Medical Sciences disagrees [29]. Concerning the study population's gender, the study found that the majority of patients are male, suggesting that more people are infected with *H. pylori*, and gender is one of the risk factors for *H. pylori* infection [30]. The findings of this investigation correspond with those of the University Hospital of Kiel [28]. And opposes Tabriz University of Medical Sciences [29]. There is a significant correlation between the ICT stool test and PCR for *H. pylori* with (P. value 0.00) and a significant correlation between the ICT stool test and *CagA* gene for *H. pylori* with (P. value 0.01) and a significant correlation between PCR for *H. pylori* and *CagA* gene for *H. pylori* with (P. value 0.01). (P. value 0.05).

Conclusion

From the above results, the PCR test with nested primers is a specific and sensitive approach for identifying *H. pylori* DNA in stool samples. The identification of *H. pylori* antigen in a stool sample is required for diagnosis of *H. pylori* infection. The age group and *H. pylori* infection had a strong link, but there was no significant correlation with age.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution Statement

AA, HA was responsible for Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision. GM, MB, BG responsible for Writing - review and editing the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available upon reasonable request from the corresponding author.

Permission and Ethical Considerations

According to research ethics, permission was obtained from all participants informed of the research objectives, and then stool samples were collected after approval by the ethical committee of medical laboratory sciences.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent to Publish

Not applicable.

Acknowledgments

The authors express gratitude to all staff of the medical laboratory sciences, Shendi University, Sudan for providing the research facilities for this study.

Bibliography

1. Graham DY., *et al*. "Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer: a randomized, controlled study". *Annals of Internal Medicine* 116.9 (1992): 705-708.

2. Humans IWGotEoCRt. "Schistosomes, liver flukes and *Helicobacter pylori*". *International Agency for Research on Cancer* 61 (1994).

3. Marshall BJ., *et al*. "Pyloric Campylobacter infection and gastroduodenal disease". *Medical Journal of Australia* 142.8 (1985): 439-444.

4. Tomb JF., *et al*. "The complete genome sequence of the gastric pathogen *Helicobacter pylori"*. *Nature* 388.6642 (1997): 539-547.

5. Rajindrajith S., *et al*. "*Helicobacter pylori* infection in children". *Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association* 15.2 (2009): 86.

6. Smolka AJ and Backert S. "How *Helicobacter pylori* infection controls gastric acid secretion". *Journal of Gastroenterology* 47.6 (2012): 609-618.

7. Torres J., *et al*. "A comprehensive review of the natural history of *Helicobacter pylori* infection in children". *Archives of Medical Research* 31.5 (2000): 431-469.

8. Abdallah TM., *et al*. "Sero-prevalence and factors associated with *Helicobacter pylori* infection in Eastern Sudan". *Asian Pacific Journal of Tropical Disease* 4.2 (2014): 115-119.

9. Burucoa C., *et al*. "Comparative Evaluation of 29 Commercial *Helicobacter pylori* Serological Kits". *Helicobacter* 18.3 (2013): 169-179.

10. Bland MV., *et al*. "The action of bismuth against *Helicobacter pylori* mimics but is not caused by intracellular iron deprivation". *Antimicrobial Agents and Chemotherapy* 48.6 (2004): 1983-1988.

11. Ermis F and Tasci ES. "Current *Helicobacter pylori* treatment in 2014". *World Journal of Methodology* 5.2 (2015): 101.

12. McNulty CA., *et al*. "Susceptibility of clinical isolates of *Campylobacter pyloridis* to 11 antimicrobial agents". *Antimicrobial Agents and Chemotherapy* 28.6 (2985): 837-838.

13. Appelmelk BJ., *et al*. "Phase Variation in *Helicobacter pylori* Lipopolysaccharide due to Changes in the Lengths of Poly (C) Tracts in α3-Fucosyltransferase Genes". *Infection and Immunity* 67.10 (1999): 5361-5366.

14. Viala J., *et al*. "Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori* cag pathogenicity island". *Nature Immunology* 5.11 (2004): 1166-1174.

15. Handbook BDP. WWW. QIAGEN. COM. Gmbh, Germany, June, (2005).

16. Makristathis A., *et al*. "Detection of *Helicobacter pylori* in stool specimens by PCR and antigen enzyme immunoassay". *Journal of Clinical Microbiology* 36.9 (1998): 2772-2774.

17. Smith SI., *et al*. "Molecular typing of Salmonella spp isolated from food handlers and animals in Nigeria". *International Journal of Molecular Epidemiology and Genetics* 2.1 (2011): 73.

18. Mitipat N., *et al*. "The prevalence of *Helicobacter pylori* infection in patients with gastrointestinal symptoms in Chon Buri, Thailand". *Southeast Asian Journal of Tropical Medicine and Public Health* 36.2 (2005): 341-346.

19. Tanih NF., *et al*. "Prevalence of *Helicobacter pylori* vacA, cagA and iceA genotypes in South African patients with upper gastrointestinal diseases". *Acta tropica* 116.1 (2010): 68-73.

20. Bjorkman DJ and Steenblik M. "Best practice recommendations for diagnosis and Management of *Helicobacter pylori*—synthesizing the guidelines". *Current Treatment Options in Gastroenterology* 15.4 (2017): 648-659.

21. Mohammed Shams Alfalah AAEA., *et al*. "Comparative Study of ICT Stool Antigen, Serum and ELISA Techniques in detection of *Helicobacter pylori* among sudanese food handler". *International Journal of Academic Health and Medical Research* 3.8 (2019): 5-6.

22. Kuipers EJ., *et al*. "Quasispecies development of *Helicobacter pylori* observed in paired isolates obtained years apart from the same host". *The Journal of Infectious Diseases* 181.1 (2000): 273-282.

23. Sicinschi LA., *et al*. "Detection and typing of *Helicobacter pylori* cagA/vacA genes by radioactive, one-step polymerase chain reaction in stool samples from children". *Journal of Microbiological Methods* 52.2 (2003): 197-207.

24. Persson C., *et al*. "*H. pylori* seropositivity before age 40 and subsequent risk of stomach cancer: a glimpse of the true relationship?" *PloS one* 6.3 (2011): e17404.

25. Jaeger EE., *et al*. "Rapid Detection and Identification of Candida, Aspergillus, and Fusarium Species in Ocular Samples Using Nested PCR". *Journal of Clinical Microbiology* 38.8 (2000): 2902-2908.

26. Bindayna KM., *et al*. "Detection of *Helicobacter pylori* cagA gene in gastric biopsies, clinical isolates and faeces". *Indian Journal of Medical Microbiology* 24.3 (2006): 195-200.

27. Salih KM., *et al*. "Prevalence of *Helicobacter pylori* among Sudanese children admitted to a specialized children hospital". *Sudanese Journal of Paediatrics* 17.1 (2017): 14.

28. Inelmen EM., *et al*. "*Helicobacter pylori* infection diagnosis in hospitalised elderly patients: the stool antigen test (HpSA) in comparison with other methods". *Aging Clinical and Experimental Research* 16.5 (2004): 349-355.

29. Saleh P., *et al*. "The association between *Helicobacter pylori* infection and rosacea". *Archives of Clinical Infectious Diseases* 13.1 (2018).

30. Porras C., *et al*. "Epidemiology of *Helicobacter pylori* infection in six Latin American countries (SWOG Trial S0701)". *Cancer Causes and Control* 24.2 (2013): 209-215.