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Association between *Helicobacter pylori* infection and upper gastrointestinal symptoms in Sudanese patients, Khartoum state- Sudan

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Abstract

Introduction: *Helicobacter pylori* is small, spiral, Gram-negative bacilli that plays a role in the pathogenesis of a number of diseases, is strongly associated with gastric cancer and peptic ulceration. The bacterium highly links to duodenal ulcer, which was classified as a group I carcinogen in 1994 by the WHO.

Objectives-This study aimed to isolate *Helicobacter Pylori* from stomach biopsy, and to assess the correlation between *H.pylori* infection and upper gastrointestinal symptoms (Epigastric pain, and hunger) and Duodenitis.

Material and Methods: This is a descriptive cross-sectional study.Out of 40 participants were included in this study. The antral mucosal biopsy specimens were obtained. According to standard microbiology procedure the specimens were cultured on Modified Brain heart Hemoglobin Urea Agar which is selective and differential media for *H. pylori*, the urease activity of *H.pylori* observed within 24 hours in this media .while, the growth were observed after 3 days following the incubation of cultured plates under microaerophilic condition provided by candle jar.

Result: *H. Pylori* was detected in 2(5%) of 40 stomach biopsy specimens. And this study revealed that there is no association between *H.pylori* infection and Epigastric pain, hunger pain and Duodenitis.

Conclusion-This study concludes that there is no association between *H.pylori* infection and upper gastrointestinal symptoms (epigastric pain and hunger) and duodenitis.

Keywords: *H.pylori***,** Stomach biopsy, Endoscopy

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Introduction: *H.Pylori (H.pylori)* are a small, spiral, Gram-negative bacillus that plays a role in the pathogenesis of a number of diseases (1). *H.pylori* is strongly associated with gastric cancer and peptic ulceration (2). The bacterium highly links to duodenal ulcer, which was classified as a group I carcinogen in 1994 by the WHO (3). Acute infection can yield an upper gastrointestinal illness with nausea and pain, vomiting and fever may also be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the *H.pylori* infection persists for years and perhaps decades or evens a lifetime (4). Long term carriage can lead to gastric adenocarcinoma and mucosa associated lymphoid tissue (MALT) and gastric lymphoma (5).There is also a growing awareness that chronic *H.pylori* infection may be associated with an increased risk of extra gastric diseases that include host iron deficiency (6), Cardiovascular, neurological, metabolic, autoimmune and dermatological diseases (7). The accurate detection of *H.pylori* is

essential for the management of patients and for the eradication of the bacterium following treatment. Since the discovery of *H.pylori*, several diagnostic methods have become available for determining the presence of *H.pylori* infection. These tests can be assessed by invasive and noninvasive methods (8). invasive (culture, histopathological examination, rapid urease test and molecular tests) (9), which require endoscopy to obtain biopsies of gastric tissues (10) and non-invasive (urea breath test, serological tests, stool culture and stool antigen/nucleic acid tests) methods may be use (9). Culture stills play a major role in the diagnostic spectrum. Culture continues to be the only test allowing for a comprehensive analysis of pathogen characteristics and susceptibility to antibiotics (11). Numerous antibiotic regiments have been evaluated for treating *H.pylori* infections (12). More success has been achieved for treatment of gastric or peptic ulcer by using a combination of bismuth ,a proton pump inhibitor (e.g., omeprazole), and one or more antibiotics (ampicillin, metronidazole, clarithromycin, tetracycline) (13). Due to the increased side effects of the treatment regimens and the development of antimicrobial resistance, a number of natural compounds have been tested as potential alternatives (14). The World Health Organization (WHO) estimated that 80% of the population in developing countries relay on traditional medicine, mostly plant based drugs, for primary health care (15). This study aimed to isolate *Helicobacter Pylori* from stomach biopsy, and to assess the correlation between *H.pylori* infection and upper gastrointestinal symptoms (Epigastric pain, Hunger and duodenitis).

Materials and Methods-This is a descriptive cross sectional study, Specimens were collected from Fedail, Military, Hospitals and ADC (Advanced Diagnostic Center) -Khartoum state Sudan. A total of forty patients were included in this study, samples of endoscopic gastric biopsies were taken from patients attending to Fedail, military hospitals and Advanced Diagnostic Center, department of gastro intestinal tract endoscopy. Data was obtained by self –administrative questionnaire. Approval to conduct this study was obtained from the Research Ethics Committee of Faculty of Medical Laboratory Sciences-Omdurman Islamic University. After explanation the study and its goal, a verbal consent was taken from the participant before proceeding with the study and collecting biopsy and stool samples.

Preparation of Modified brain heart hemoglobin urea agar (MBHUA) media- This media was modified by Alkhidir (16), Modified Brain heart Urea Agar (MBHUA) was prepared by adding 52 gm of brain heart agar (Hi media - India), 20 gm of urea base (Hi media - India), and 0.0012 gm of phenol red as an indicator (Hi media - India) to 740 ml of distilled water and sterilized by autoclaving at 121ºC for 15minutes, then cooled to 50-55ºC. 10 ml of antibiotic solution was added (1 mg of vancomycin, 5 mg of trimethoprim, and 5mg of amphotericin B in 10 ml sterile distilled water), and 3gm of urea crystal (Hi media - India) was added and then mixed thoroughly and poured into sterile disposable Petri dishes (17).

Sample collection and transportation-Stomach biopsy specimens were collected in plain container

contain 2 ml of normal saline and transported in sterile plain container containing sterile normal saline or phosphate buffer saline at 4^0C.

Sample processing and culture-The 40 stomach biopsy specimens were centrifuged in normal saline or phosphate buffer saline at 3000rpm for 2minutes,then supernatant was discarded and two to three drops of sediment was cultured on modified media (MBHUA) using sterile wire loop by ordinary method (primary, secondary, tertiary and zigzag) under a septic condition.

All plates incubated at 37^0C in microaerophilic conditions using candle jar up to 5 days and observed for growth and change in the color of the medium daily, growth was observed after 3 days. Colonies showed change in the color of the medium to pink (indicating rise in the PH of the medium due urease production with subsequent breakdown of urea into ammonia and carbon dioxide).

Then purified colonies were identified based on Gram stain reaction, and biochemical tests (catalase, oxidase, and urease). The data analyzed by using Statistical Package for Social Sciences (SPSS) program**.**

Result: Among 40 stomach biopsy obtained from participants by upper gastrointestinal endoscopy, only two (5%) biopsies showed growth of *H.pylori*, and the rest (95%) showed no growth, (Figure 1). According to the gender equal percentage of growth of *H.pylori* from stomach biopsies was observed, (Table 1).

Out of 40 Endoscopic biopsy enrolled in this study, divided into three age groups; less than 30 years, 30-60 years, and less than 60 years. And growth of *H.pylori* was found in 0%, 2.5%, and 2.5% respectively. Evaluation of culture result according to the age showed statistically insignificance correlation with the age, (*P* value=0.5), (Table 2).

In patients with Epigastric pain the percentage of *H.pylori* growth was 5%, and 70% showed no growth. In those without epigastric pain the percentage of growth was 0%, and no growth was 25%, (Table 3).

In patient with hunger the percentage of growth of *H. pylori* was 2.5%, and no growth was 47.5%, and in those without of hunger showed similar result, (Table 3).

In in patients with Duodenitis frequency of *H. pylori* growth was 0%, and no growth was 25%. And in those without Duodenitis growth percent of *H.* pylori was 5%, and 70% showed no growth.

Growth of *H. pylori* was observed in 5% of patients had past history of *H.pylori* infection, and no growth observed in 42.5% of them. No growth observed patient had not past history of *H. pylori* infection, (Table 4).

In patients had antibiotic treatment the percentage of growth was 5%, and no growth was 40%, and there was no growth observed in those not use antibiotic treatment.

According to P value of different variable there was insignificance when analyzed statistically, (Table 4).

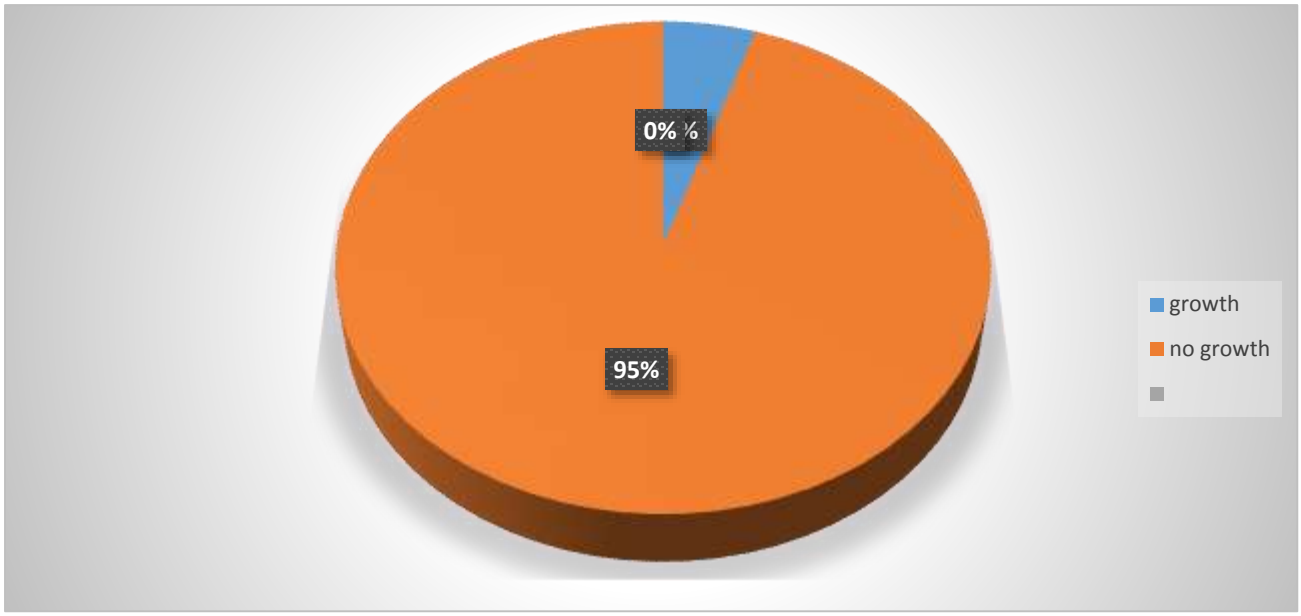

Figure 1: frequency of growth of H.pylori in Stomach biopsy samples

Table 1: frequency of growth of *H.pylori* among gender in Stomach biopsy samples

		culture result		*P* value
		Growth	No growth	
Sex	Male	2.5%	47.5%	1
	Female	2.5%	47.5%	
Total		5%	95 %	

Table 2: comparison of culture result with age group (biopsy)

		culture result		*P* value
		growth	No growth	
Age	less than 30 years	0%	30%	0.5
	30-60 years	2.5%	45%	
	more than 60 years	2.5%	20%	
	Total	5%	95%	

Table 3: comparison of culture result with symptoms

		culture result		*P* value
		Growth	No growth	
Epigastric pain	Yes	5%	70%	0.4
	No	0%	25%	
	Total	5%	95%	
Hunger	Yes	2.5%	47.5%	1
	No	2.5%	47.5%	
	Total	5%	95%	
Duodenitis	Yes	0%	25%	0.4
	No	5%	70%	
	Total	5%	95%	

Table 4: comparison of culture result with Past history of *H.pylori* infection and previous antibiotic use

		Culture result		P value
		Growth	No growth	
Past infection	Yes	5%	42.5%	0.1
	No	0%	52.5%	
	Ttal	5%	955	
Previous antibiotic use	Yes	5%	40%	0.1
	No	0%	55%	
	Ttal	5%	955	

Discussion:

The primary isolation of *H. pylori* from biopsy specimens is difficult process. This may be due to fastidious nature of *H. pylori* and a number of factors that are hard to control (patchy distribution of the organism on gastric mucosa, contamination of biopsy forceps, ingestion of anesthetic, presence of oropharyngeal flora, loss of the viability of the organism during transportation, etc.) (18).

Multiple effort from multiple laboratories have been unsuccessful and the optimal condition to recover *H. pylori* from stool still not known (19). The frequency of *H.pylori* growth among gender is equal.

The patients were classified into 3 age groups, less than 30 years, 30-60 years, more than 60 years. In our study there is no association between age ,gender, and culture result, this result agree with study done by Uszczynska K *et* al in Poland, Akbar DH and Eltahawy ATA in Saudi Arabia, and Petrovic M *et al* in Serbia (20-22), and study done by Maha *et al,* Saudi Arabia, aimed to estimate the prevalence of *H.pylori* among patients suffering from upper gastrointestinal symptoms, *H.pylori* status in patients was determined by histology, rapid urease test, and ELIZA. The study showed that there was a significance correlation between age, gender and *H.pylori* infection (23) this may be due to use of more than one test for detection of *H.pylori* ,which are more sensitive than culture, increasing chance for detection of the organism.

Our study revealed that there is no significance association between symptoms of epigastric pain, hunger, and duodenitis and *H.pylori* infection. In study done by Rosenstock, *et al* in Denmark, that aimed to assess the relation between *H.pylori* infection and gastro-intestinal symptoms and syndromes, random sample size was 3589 and Werdmuller, *et al* in Australia, sample size was 404 reported that there was association between epigastric pain and *H.pylori* infection, this may be due to variation in sample size and geographical area (24, 25).

The present study showed that there is no association between past infection, Previous antibiotic use and positive growth culture.

Patients with past infection with *H.pylori* and use antibiotic treatment showed positive *H.pylori* culture this may be due to patients were not complete the course of treatment, or patients had been recovered from the past infection and this is new infection, or patient was infected with antibiotic resistant *H.pylori* strain.

Conclusion-This study concluded that, there is no

association between upper gastrointestinal symptoms (epigastric pain and hunger), duodenitis, past *H.pylori* infection, previous antibiotic use and *H.pylori* infection.

References

1. Kouitcheu Mabeku LB, Eyoum Bille B, Tchouangueu TF, Nguepi E, Leundji H. Treatment of Helicobacter pylori infected mice with Bryophyllum pinnatum, a medicinal plant with antioxidant and antimicrobial properties, reduces bacterial load. Pharmaceutical biology. 2017;55(1):603-10.

2. Wang YC, Huang TL. High-performance liquid chromatography for quantification of plumbagin, an anti-Helicobacter pylori compound of Plumbago zeylanica L. Journal of chromatography A. 2005;1094(1-2):99-104.

3. Wang YC. Medicinal plant activity on Helicobacter pylori related diseases. World journal of gastroenterology. 2014;20(30):10368-82.

4. Jawetz M, Adelberg, editor. Medical Microbiology. 27 ed: McGraw Hill Education; 2016.

5. Kabir S. Detection of Helicobacter pylori in faeces by culture, PCR and enzyme immunoassay. Journal of medical microbiology. 2001;50(12):1021-9.

6. Flores SE, Aitchison A, Day AS, Keenan JI. Helicobacter pylori infection perturbs iron homeostasis in gastric epithelial cells. PloS one. 2017;12(9):e0184026.

7. Lewinska A, Wnuk M. Helicobacter pylori-induced premature senescence of extragastric cells may contribute to chronic skin diseases. Biogerontology. 2017;18(2):293-9.

8. Aktepe OC, Ciftci IH, Safak B, Uslan I, Dilek FH. Five methods for detection of Helicobacter pylori in the Turkish population. World journal of gastroenterology. 2011;17(47):5172-6.

9. Cagdas U, Otag F, Tezcan S, Sezgin O, Aslan G, Emekdas G. [Detection of Helicobacter pylori and antimicrobial resistance in gastric biopsy specimens]. Mikrobiyoloji bulteni. 2012;46(3):398-409.

10. Russmann H, Kempf VA, Koletzko S, Heesemann J, Autenrieth IB. Comparison of fluorescent in situ hybridization and conventional culturing for detection of Helicobacter pylori in gastric biopsy specimens. Journal of clinical microbiology. 2001;39(1):304-8.

11. Gonzalez-Vazquez R, Herrera-Gonzalez S, Cordova-Espinoza MG, Zuniga G, Giono-Cerezo S, Hernandez-Hernandez JM, et al. Helicobacter pylori: detection of iceA1 and iceA2 genes in the same strain in Mexican isolates. Archives of medical research. 2012;43(5):339-46.

12. Patrick R. Murray P, Ken S. Rosenthal P, George S. Kobayashi P, Micheal A. Pfaller M. Medical Microbiology. 2002(Four).

13. Parija SC. Text book of Microbiology and Immunology. 2012.

14. Mandalari G, Bisignano C, Cirmi S, Navarra M. Effectiveness of Citrus Fruits on Helicobacter pylori. Evidence-based complementary and alternative medicine : eCAM. 2017;2017:8379262.

15. Yagoub SO. Anti-Microbial Activity of *Tamaridus indica* and *Adansonia digitata* Extracts Against E. coli Isolated from Urine and Water Specimens. Research Journal of Microbiology. 2008;3(3):193-7.

16. Abdulazeem Abdulsalam Ibrahim Alkhidir, Esraa Hassan Mohammed Ibrahium, Fatima Suliman Ali Ahmed, Lubaba Bashieer Alameen Bashieer, Mashaer Hassan Mohammed Hessen, Manal Bashieer Alameen Bashieer, et al. Detection of Helicobacter Pylori from Stomach Biopsies: A Rapid Culture Method. Med Forum. 2022;33(10):10.

17. Alkhidir AAI, Abusalif KO, Nasseir MEO, Elhussain KMK. Helicobacter pylori: Rabid culture method and The antibacterial effect of Moringa and Ginger against H. pylori. 2017:5.

18. Piccolomini R, Di Bonaventura G, Festi D, Catamo G, Laterza F, Neri M. Optimal combination of media for primary isolation of Helicobacter pylori from gastric biopsy specimens. Journal of clinical microbiology. 1997;35(6):1541-4.

19. Dore MP, Osato MS, Malaty HM, Graham DY. Characterization of a culture method to recover Helicobacter pylori from the feces of infected patients. Helicobacter. 2000;5(3):165-8.

20. Leszczynska K, Namiot A, Namiot Z, Leszczynska JK, Jakoniuk P, Chilewicz M, et al. Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. Advances in medical sciences. 2010;55(2):161-6.

21. Petrovic M, Artiko V, Novosel S, Ille T, Sobic-Saranovic D, Pavlovic S, et al. Relationship between Helicobacter pylori infection estimated by 14C-urea breath test and gender, blood groups and Rhesus factor. Hellenic journal of nuclear medicine. 2011;14(1):21-4.

22. Akbar DH, Eltahawy AT. Helicobacter pylori infection at a university hospital in Saudi Arabia: prevalence, comparison of diagnostic modalities and endoscopic findings. Indian journal of pathology & microbiology. 2005;48(2):181-5.

23. Abo-Shadi MA, El-Shazly TA, Al-Johani MS. Clinical,Endoscopic,Pathological and Serological finding ofH.pylori Infection in Saudi Patients with UpperGastrointestinal Diseases. British Journal of Medicine & Medical Research. 2013;3(4):1109-24.

24. Rosenstock S, Kay L, Rosenstock C, Andersen LP, Bonnevie O, Jorgensen T. Relation between Helicobacter pylori infection and gastrointestinal symptoms and syndromes. Gut. 1997;41(2):169-76.

25. Zipser RD, Parikh MV. Is repeat testing needed for Helicobacter pylori? The Journal of the American Board of Family Practice. 2000;13(6):449-50.