Patient and Microbial Factors Affecting Culture of Helicobacter Pylori

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Abstract

Background: Helicobacter pylorus has been identified as a major cause of peptic ulcer disease, risk factor for gastric cancer and mucosa associated lymphoid tissue (MALT) lymphoma. There is an emerging increase in antimicrobial resistance and subsequently failing empiric *H. pylori* eradication therapies which increases need to asses antibiotic susceptibility for H. pylori in every country, this will be done after its culture. Culturing of H. pylori is affected by multiple factors. **Aim of the work:** To Identify factors affecting H. pylori culture. **Patients and Methods:** A group of 134 adult patients with upper gastrointestinal complaints were recruited excluding patients who received PPI in last 2 weeks and Antibiotic in last 4 weeks. Upper GIT endoscopy was done, biopsies were collected; histopathological examination and culture of H. pylori were done, we studied the relation between culture result, patients and bacterial factors. **Results:** Out of 134 studied biopsies, 20 had H. pylori culture positive (14.9%), the bacillary form of H. pylori was more cultivable (9/24, 37.5%) (P = 0.008). Positive culture results were associated with moderate infestation by H. pylori (14/20, 70%). Diabetes mellitus was associated with positive culture result (5/20, 25%) with (P = 0.04). Only one case of culture positive H. pylori had previous history of H. pylori treatment (1/20, 5%). **Conclusion:** H. pylori culture is affected by multiple factors besides technical factors include form of H. pylori organism and degree of infestation of tissue by H. pylori, other factors like DM and previous H. pylori treatment of the patient.

Keywords: H. pylori, culture, factors, D.M.

Abbreviations: (H. pylori= Helicobacter pylori, DM= Diabetes Mellitus, P= P-Value)

Introduction:

Helicobacter pylori (H. pylori) infection is highly prevalent in the human population and may lead to severe gastrointestinal pathology including gastric and duodenal ulcers, mucosa associated tissue lymphoma and gastric adenocarcinoma (1)**.**

As *H. pylori* easily develops drug resistance to single antibiotics, combination therapy of several antibiotics is recommended. Combination of antibiotics used in therapy should depend on local drug resistance rates estimated in the respective country (2)**.**

H. pylori culture and antimicrobial susceptibility testing is carried out to predict antibiotic treatment outcome and guide clinicians in their choice of therapy (3)**.**

Several diagnostic methods are available for detecting *H. pylori* infections. They can be classified as invasive and non-invasive methods depending on the need to gastric biopsy from the patient or not. For *H. pylori* detection, endoscopy is employed in combination with histology and/or culture from the gastric biopsy specimen. The major limitation of endoscopic examination is its relative invasiveness and that only a small portion of the gastric mucosa can be explored. Therefore, assessment of multiple gastric biopsy specimens is necessary to provide a global picture of *H. pylori* infection in the stomach (4)**.**

Successful isolation and cultivation of *H. pylori* from gastric biopsy specimens is a challenging task that is affected by a number of factors like the quality of the clinical specimen, occurrence of microbial commensal flora in clinical specimens, time interval between sampling and culture and inappropriate transport conditions (temperature, duration of air exposure, *etc*.). Furthermore, *H. pylori* culture requires highly trained laboratory personnel and takes up to 7 d until samples can be reported as negative and up to 2 weeks until *H. pylori* has grown. *H. pylori* culture from gastric biopsy specimens typically has a sensitivity greater 90% and a specificity of 100%, when performed under optimal conditions (5)**.**

Host factors like high activity of gastritis, low bacterial load, bleeding, alcohol drinking, and use of H_2- receptor antagonists, PPI, antibiotics have adverse effect on culture positive rate. These medications, except for antibiotics which should be avoided at least 4 weeks, were also suggested to be avoided 2 weeks before culture (6)**.**

With the increasing prevalence of antibiotic resistance, culturing is still a reliable method for managing *H. pylori* treatment failure as well as surveying antibiotic resistance in population-based studies (7) **.**

Aim of the work: To identify factors affecting H. pylori culture.

Patients and Methods:

This cross section study was conducted on 134 adult patients with dyspepsia who attended the endoscopy unite of Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital between October 2018 and October 2020. The study protocol was approved by Research Ethics Committee of Benha faculty of Medicine. All patients gave informed consent before inclusion in the study.

We excluded patients who have received PPI during the last 2 weeks or anti-microbial therapy during the last 4 weeks before endoscopy. We excluded also patients who refused to give informed consent.

Demographic date (like age, sex, and smoking habit), drug history to previous H. pylori treatment and non-steroidal anti-inflammatory drugs and past history to diseases like DM and Hypertension were taken.

A venous blood sampling was taken for complete blood count (CBC).

Upper gastrointestinal tract endoscopy was done using disinfected upper gastrointestinal video scope (Olympus flexible endoscope) by immersion in 2.2% Glutaraldehyde solution for 20 minutes at 25°C (room temperature**),**then washed with water (8).

Good preparation of the patient by fasting for at least 8 hours before the procedure.

The patient lies in the left lateral position with flexed neck then receives Metazolam 10 mg (intravenous) for sedation.

Complete examination of the esophagus, stomach and the duodenum down to the second part of the duodenum stressing on signs suggesting H. pylori infection e.g. inflammation, erosions, ulcers or masses.

We took 4 gastric biopsies. Two biopsy specimens were preserved in a container using diluted formalin solution for histopathological examination using Haematoxylin-Eosin (H&E) stain, examined for the presence of H. pylori , presence of gastritis ,degree of activity and chronicity of gastritis, presence of atrophy, intestinal metaplasia, dysplasia or lymphoid follicles according to Updated Sydney Classification (9) . We used also modified Giemsa stain to confirm the diagnosis of H. pylori infection.

Another two biopsies were cultured after grounding using sterile glass rods under aseptic condition. Culture media was prepared as a suspension of 39 g of Columbia blood agar base dissolved in 1L of distilled water, then boiled to dissolve completely and sterilized by autoclaving at 121˚c for 15 minutes then cooled and 5% sterile defibrinated blood was added. Two ml of distilled water was added to H. pylori selective supplement vial, mixed gently, added to 500 ml of Columbia blood agar base with defibrinated blood then mixed well and dispensed into sterile petri dishes. A portion of the grounded material for each sample was inoculated into freshly prepared Columbia blood agar culture petri dishes. All plates were incubated at 37˚c under microaerophilic conditions using the gas pack jar and were inspected within 3-4 days.

H. pylori colonies (Figure1) were identified by Urease test, characterized colonies morphology **(**circular, convex, translucent colonies about 2 mm in diameter), Oxidase positive test, Catalase positive test and microscopic examination.

Statistical Methods:

Statistical analysis:

Sensitivity and specificity of culture method for H. pylori diagnosis were calculated. Comparison between culture positive and culture negative patients were done regarding demographic, clinical, endoscopic and histopathological variables.

SPSS program was used to get mean, SD and significance, two tailed student -test or Man Whitney test was used for comparison of continues variables. Chi Square test or Fisher's exact test was used for dichotomous or categorical variables.

Results:

Out of the 134 dyspeptic adult patients included in this study, there was 56 males (41.8%) and 78 females (58.2%). Abdominal pain, heart burn, nausea, vomiting, hematemesis and melena were the main presenting symptoms followed by bloating, diarrhea, easy fatigability and loss of weight (Table 1).

Endoscopy was done for the enrolled patients. We found that 71(53%) patients had gastric erosions, 41 (30.6%) had endoscopic mucosal granularity, and 21 (15.7%) had gastric ulceration (Table 2). We found that gastric ulceration had a significant association with male gender (P = 0.005), clinical presentation with pallor (P = 0.003) and had tendency for significant association with clinical presentation of hematemesis and/or melena (P = 0.07), and smoking (P = 0.1).

And what is noteworthy that presence of gastric ulceration had a negative association with previous treatment of H. pylori. We have 21 patients with gastroduodenal ulcers, none of them had previously treated for H. pylori (0/21, 0%). We have 113 patients without gastroduodenal ulcers, 18 of them (15.9%) had previously received H. pylori treatment (P = 0.05).

Histopathological examination detected H. pylori organism in 121 from 134 (90.3%) of our patients. H. pylori was seen in the form of cocci in 50/121 (41.3%), cocco-bacilli in 47/121 (38.3%) and bacilli in 24/121 (20.4%).

In spite of small number of biopsies with gastric mucosal atrophy noticed by histopathology, there was tendency of association between culture positive and gastric mucosal atrophy (p- value = 0.06).

The other biopsies obtained were subjected to culture for H. pylori. Out of the 134 studied biopsies, 20 had H. pylori culture positive (14.9%). All biopsies that tested H. pylori negative in histopathology were found culture negative. All biopsies that tested H. pylori negative in histopathology were found culture negative. H. pylori culture had 100% specificity, but 20 out of 121 histopathology positive had culture positive (20/121) 16.5 % sensitivity (Table 3). The bacillary form of H. pylori was more cultivable (9/24, 37.5%) in comparison to cocci (6/50, 12%) and cocco-bacilli (5/47, 10.6%) (P = 0.008) (Table4), more positive results were with moderate infestation by H. pylori (14/20, 70%) (Table5). Diabetes mellitus (D.M) was associated with positive culture results (5/20, 25%) with (P = 0.04).Only one case of culture positive H. pylori had previous history of H. pylori treatment (1/20, 5%) (Table6).

Table 1: Demographic and clinical variables of our studied group:

Variable	Total (N= 134)
Male gender	56 (41.7%)
Age, Years (Mean ±SD)	42.2± 14.4
Smoker	33 (24.6%)
Abdominal pain	129 (96.3%)
Nausea and vomiting	74 (55.2%)
Bloating	42 (31.3%)
Heart burn	112 (83.6%)
Diarrhea	9 (6.7%)
Easy fatigability	9 (6.7%)
Loss of weight	18 (13.4%)
Hematemesis and/or Melena	53 (39.6%)
Previous H. pylori treatment	18 (13.4%)
NSAID intake	52(38.2%)
DM	15 (11.2%)
Pallor	62 (46.3%)
Abdominal tenderness	113 (84.3%)

Table 2: Description of the studied group regarding endoscopic finding:

Variable	**Total (N= 134)**
Endoscopic Granularity	41 (30.6%)
Endoscopic Erosions	71 (53%)
Endoscopic Ulceration	21(15.7%)

Table 3: Calculation of sensitivity and specificity of H. pylori culture regarding histopathology results of presence of H. pylori:

	Histopathology positive	**Histopathology negative**	**Total**
Culture positive	20	0	20
Culture negative	101	13	114
Total	121	13	134

- So sensitivity of H. Pylori culture = 20/121=16.5%
- Specificity of H. pylori culture =13/13=1=100%

Table 4: Comparison between culture positive and negative regarding H. pylori morphology:

Morphology	**culture positive (T=20)**	**Culture negative (T=101)**	**p- value**
Cocci	6 (30%)	44(43.6%)	0.008
Bacilli	9 (45 %)	15(14.9 %)	
Cocco-bacilli	5 (25 %)	42(41.6 %)	

Table 5: Comparison between culture positive and negative regarding Heaviness of H. pylori infection:

Heaviness of infection	**culture positive (T=20)**	**Culture negative (T=101)**	**p- value**
Mild	6 (30 %)	52 (51.5%)	0.079
Moderate	14 (70 %)	49 (48.5%)	

Table 6: Comparison between culture positive and negative regarding previous history of disease and treatment:

History of chronic illness	**Culture positive (T=20)**	**Culture negative (T=114)**	**p-value**
DM	5 (25 %)	10 (8.8 %)	0.04
Previous H. pylori treatment	1 (5%)	17 (14.9 %)	0.2

(Figure1) H. pylori colonies

Discussion:

H. pylori has been recognized as one of the main pathogenic factors in the occurrence and development of gastrointestinal diseases such as chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. It has been categorized as the class I carcinogen by World Health Organization (10).

Since the discovery of *H. pylori*, bacterial culture has been used as routine diagnostic test, being considered the gold standard. Currently, the Maastricht-4 Consensus Report recommends *H. pylori* culture for performing antibiotic susceptibility testing if primary resistance to clarithromycin is higher than 20% or after failure of second-line treatment (11).

In this study, the sensitivity of culture method in detection of H. pylori in comparison to histopathology was low (16.5%), however specificity was 100%. Other studies reported a higher sensitivity figures. A study, conducted in Philippines-philippine General Hospital in 2004, revealed 30 % sensitivity (12). Another study detected H. pylori culture sensitivity 29.3% in Chinese patients (13). The difference in culture sensitivity may be attributed to variation in the isolation of the organism by culture because success rates depend on the technical expertise of the

microbiology laboratory, adequacy of tissue sampling, transport media and time, culture media and incubation period.

We found that biopsies that have bacillary form of H pylori during histopathological examination are more likely to have culture positive than other forms. This observation was reported many years ago in a study which found that bacillary form was colonized (100%-6 /6) but coccoid form was not colonized in any of inoculated piglets. He considered coccoid form of H. pylori as a degenerative nonviable non-culturable morphologic phase (14).

As regard the association between H. pylori culture positive results and diabetes mellitus, there was significant statistical association between culture positive and D.M which is similar to results from a study which found that D.M increased incidence of H. pylori-colonization, this may be due to the reduced gastric motility and chemical changes in gastric mucosa following non-enzymatic glycosylation processes (15) . Other explanation is that H. pylori is more prevalent in diabetic patients than healthy individuals or nondiabetic patients (16).

As regard the association between H. pylori culture positive results and atrophic gastritis detected by histopathological examination, there was significant association between culture positive and atrophic gastritis because H. pylori infection is significantly associated with the presence of gastric mucosal atrophy (17).

One of the drawbacks of our study is that our estimation for sample size was based on prevalence of H. pylori and assumption of 50% sensitivity for culture method. Unfortunately, culture method in our study had a lower sensitivity

Conclusion:

- H. pylori culture is a definitive method for diagnosis of H. pylori but with low sensitivity.
- H. pylori culture is affected by multiple factors besides technical factors include bacterial factors like form of H. pylori organism and degree of infestation of tissue by H. pylori, and host factors like D.M and previous H. pylori treatment of the patient.

References:

1. Pohl D, Keller PM, Bordier V, Wagner K. Review of current diagnostic methods and advances in Helicobacter pylori diagnostics in the era of next generation sequencing. World J Gastroenterol. 2019 Aug;25(32):4629–60.

2. Shao Y, Lu R, Yang Y, Xu Q, Wang B, Ye G. Antibiotic resistance of Helicobacter pylori to 16 antibiotics in clinical patients. J Clin Lab Anal. 2018;32(4).

3. Tominaga K, Watanabe T, Fujiwara Y, Higuchi K, Arakawa T. [Morphological investigation of H. pylori and its culture method]. Nihon Rinsho. 2001 Feb;59(2):296–300.

4. Bayerdörffer E, Oertel H, Lehn N, Kasper G, Mannes GA, Sauerbruch T, et al. Topographic association between active gastritis and Campylobacter pylori colonisation. J Clin Pathol. 1989;42(8):834–9.

5. Hirschl AM, Makristathis A. Methods to detect Helicobacter pylori: from culture to molecular biology. Helicobacter. 2007 Nov;12 Suppl 2:6–11.

6. Leszczyńska K, Namiot A, Namiot Z, Leszczyńska JK, Jakoniuk P, Chilewicz M, et al. Patient factors affecting culture of Helicobacter pylori isolated from gastric mucosal specimens. Adv Med Sci. 2010;55(2):161–6.

7. Wang Y-K, Kuo F-C, Liu C-J, Wu M-C, Shih H-Y, Wang SSW, et al. Diagnosis of Helicobacter pylori infection: Current options and developments. World J Gastroenterol. 2015 Oct;21(40):11221–35.

8. Rutala WA, Weber DJ. Disinfection and sterilization in healthcare facilities. Bennett Brachman's Hosp Infect Sixth Ed. 2013;(May).

9. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis: the updated Sydney system. Am J Surg Pathol. 1996;20(10):1161–81.

10. Du L-J, Chen B-R, Kim JJ, Kim S, Shen J-H, Dai N. Helicobacter pylori eradication therapy for functional dyspepsia: Systematic review and meta-analysis. World J Gastroenterol. 2016;22(12):3486.

11. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon ATR, Bazzoli F, et al. Management of Helicobacter pylori infection—the Maastricht IV/Florence consensus report. Gut. 2012;61(5):646–64.

12. Destura R V, Labio ED, Barrett LJ, Alcantara CS, Gloria VI, Daez MLO, et al. Laboratory diagnosis and susceptibility profile of Helicobacter pylori infection in the Philippines. Ann Clin Microbiol Antimicrob. 2004;3(1):1–6.

13. Tang X, Chen X, Shen Y, Yang T, Hu R, Debowski AW, et al. Primary antibiotic resistance of Helicobacter pylori among a Chinese Tibetan population. Future Microbiol. 2020;15(14):1353–61.

14. Eaton KA, Catrenich CE, Makin KM, Krakowka S. Virulence of coccoid and bacillary forms of Helicobacter pylori in gnotobiotic piglets. J Infect Dis. 1995 Feb;171(2):459–62.

15. Perdichizzi G, Bottari M, Pallio S, Fera MT, Carbone M, Barresi G. Gastric infection by Helicobacter pylori and antral gastritis in hyperglycemic obese and in diabetic subjects. New Microbiol. 1996 Apr;19(2):149–54.

16. Hosseininasab Nodoushan SA, Nabavi A. The Interaction of Helicobacter pylori Infection and Type 2 Diabetes Mellitus. Adv Biomed Res. 2019;8:15.

17. Myint T, Shiota S, Vilaichone R, Ni N, Aye TT, Matsuda M, et al. Prevalence of Helicobacter pylori infection and atrophic gastritis in patients with dyspeptic symptoms in Myanmar. World J Gastroenterol. 2015 Jan;21(2):629–36.

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