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CLINICAL SIGNIFICANCE OF VARIOUS DIAGNOSTIC TECHNIQUES AND EMERGING ANTIMICROBIAL RESISTANCE PATTERN OF HELICOBACTER PYLORI FROM GASTRIC BIOPSY SAMPLES

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ABSTRACT

BACKGROUND: There is no single technique that can meet the criteria in identification of Helicobacter pylori. The diagnosis is important as antimicrobial resistance is frequently observed and associated with treatment failure. The present study was conducted to evaluate diagnostic tests for identification of H.Pylori and to assess their antimicrobial resistance pattern.

MATERIALS AND METHODS: Biopsies of gastric tissues from 100 patients with disorders of upper gastrointestinal tract were studied for detection of H.Pylori by various methods like culture, H & E staining and urease test. Antimicrobial susceptibility testing was carried out by Kirby Bauer's disc diffusion method.

RESULTS:- Out of 100 patients H.Pylori was detected by rapid urease test, H and E staining and culture in 28%, 14.5%, and 2.5% cases respectively. H and E staining was taken as the gold standard. Senensitivity of Urease test was 76.6%, and culture 13.3%. Specificity of Urease was 81.7% in comparision with culture which showed 99.4% specificity.Metronidazole (05) showed high level of resistance followed by amoxicillin (03) and norfloxacin (03). Tetracycline,erythromycin, levofloxacin and cotrimoxazole showed one resistance each to H Pylori.

CONCLUSION:- H and E is taken as the gold standard according to CDC.Urease test is better screening procedure than culture. H.Pylori resistance to metronidazole in our zone was highest. This is due to general and extensive use of metronidazole for other infectious diseases. Our study suggests need for a systematic approach to determine antibiogram of strains before considering the drug regimens

KEYWORDS : Gastric biopsy, H and E staining, H.pylori, metronidazole, urease test.

INTRODUCTION

Helicobacter pylori (H.pylori) is commonly associated with chronic gastritis in more than 50% of the people worldwide. In developing countries 70-90% of the population carries H pylori[12] . Many methods invasive and non-invasive have been developed for the diagnosis of H.pylori infection [3]. Invasive methods requiring endoscopic evaluation include bacteriologic culture,histopathologic studies , smear examination like Grams and giemsa stain, rapid urease test and molecular studies. Non invasive methods include serologic testing, urea breath testing antigen testing in the stool [4]. In spite of available diagnostic methods to detect H.pylori, there is no single technique that can meet the criteria for acceptable sensitivity and specificity in identification of the bacterium [1]. Therefore various diagnostic methods are recommended in a combination of two or more to meet diagnostic criteria [5,6].

Although culture is needed for antimicrobial susceptibility, it is difficult to isolate,requires an enriched transport medium, is expensive and time consuming[4]. Histopathology is considered as the standard method for the diagnosis of H.pylori infection, but the reliability depends on the number and site of specimens collected [7]. A further limitation of uses of histopathology with regard to sensitivity Specificity is the quality of the biopsies. If the biopsy is too small, poorly oriented or inappropriately fixed or stained, detection of H pylori may not be possible [4]

The specific diagnosis is important because clinical experience has demonstrated that H.pylori infection is not easy to cure[8]. (Triple therapy combining a proton pump inhibitor with two antibiotics like clarithromycin,metronidazole, or amoxicillin, represents the regimen for eradication. Treatment failure occurs due to resistant strains particularly metronidazole [8]. The resistance of H.pylori to the antimicrobials is a growing problem . In developed countries metronidazole resistance is found in 10-15% of adult H.pylori infected patients. Whereas in developing countries almost all strains are resistant to the antimicrobial agent.[9] Antibiotic resistance frequently causes failure of eradication of H.pylori[9]

Therapeutic regimens followed currently are mostly based on either insufficient data or obtained from other geographically un-related region. In the Indian population the prevalence of H.pylori resistance is very high to metronidazole (77.9%), followed by clarithromycin (44.7%). Amoxicillin (32.8%) and ciprofloxacin showed least resistance (1-4%).[10]. This study was done to evaluate the antimicrobial resistance pattern in this region.

Each of the above methods has advantages and disadvantages and non can be considered as a single gold standard. A combination of endoscopic biopsy based methods usually gives the most reliable diagnosis[11]. Thus this study is aimed to evaluate diagnostic like urease test, culture and Hematoxyline and Eosin(H and E) staining and to assess the antimicrobial resistance pattern.

MATERIALS AND METHODS

One hundred patients having disorders of the upper gastrointestinal tract were studied by video-endoscopy. Patients who had taken antibiotics , H_2 blockers or proton pump inhibitors 24 hrs prior to endoscopy were excluded. Patients with dyspepsia but a normal endoscopic appearance were classified as non-ulcer dyspepsia . Others who had oedema or redness of the gastric mucosa which was histologically confirmed as gastritis were included in the chronic gastritis group. Informed consent and ethical clearance from the Institutional Ethical Committee were obtained.

Two biopsy specimens of gastric tissue from each patient were collected by gastroenterologist during endoscopy . One biopsy was transported to the microbiology laboratory within half to one hour in the brain heart infusion (BHI) with glycerol. They were submitted for rapid urease test and culture. The other biopsy for histopathology examination was transported in 10% buffered formalin, routinely processed and embedded in paraffin. The slides were stained with routine H and E stain . The H.pylori were identified as curved rods on the luminal surface of the gastric epithelial cells.

Culture of H.pylori from biopsy sample:-The biopsy specimen was inoculated onto Columbia blood agar plates supplemented with 10% sheep blood,Campylobacter growth supplement and Campylobcter selective supplement , incubated in CO_2 jar with multiple wax candles lighted to create the micro-aerophilic atmosphere. While closing the jar each time, petroleum jelly was put on the side of the rim of the jar and then the jar was closed tightly. The jar was kept at 37 ^{0}C for 5-7 days [12]. The bacterial colonies were identified on the basis of colony morphology, Grams staining, positive oxidase, catalase and urease reactions. Bacteria were subcultured into two different media Columbia blood agar with supplements and brain heart infusion broth with 20% glycerol.

Rapid urease test

This was done using a urea broth prepared and standardised in our set

up where the concentration of urea was increased to double the amount to increase the sensitivity of the test.

Antimicrobial susceptibility testing of the isolate

A total of 03 isolates of H.pylori subcultured were tested for antimicrobial susceptibility using Kirby-Bauer disc diffusion method on Muller-Hinton agar plate supplemented with 10% sheep blood. A standard inoculum of H.pylori culture was suspended in BHI broth. The turbidity was adjusted equal to McFarland 3. The inoculum was seeded onto Muller-Hinton blood agar plate using sterile cotton wool swab, antibiotic discs with the following drug content; Metronidazole (5 ug). Amoxicillin (10 ug). Tetracycline (30 ug), erythromycin (15 ug), levofloxacin (5 ug), norfloxacin (5 ug), cotrimoxazole (10 ug) were placed on the plates. The plates were incubated at 37^{0}C in CO_2 jar for 3-4 days . The results were interpreted as per Clinical Laboratory Standards Institute (CLSI)2011 guidelines .[13]

RESULTS:- A total of 100 patients 76 males and 24 females with various upper Gastro intestinal disorders were included in the study. H.pylori was detected by rapid urease test in 28 (28%) cases where urease was positive in 24 (24.21%) cases with chronic gastritis, and 4 (80%) cases with peptic uler . By histopathology slides stained with H & E in 15 (14.5%) cases where 12 (12.11%) cases had chronic gastritis and 03 (60%) cases had peptic ulcer and culture in 3 (2.5%) cases where 01 (0.25%) case had chronic gastritis and 02 (40%) had peptic ulcer as shown in Table 2. H and E was taken as the gold standard. Sensitivity of urease test was 76.6% and of culture 13.3%. Specificity of urease was 81.7% in comparison with culture which showed 99.4% specificity.

Out of three isolates subjected to antimicrobial susceptibility testing, metronidazole (3) showed high level of resistance followed by amoxicillin (2) and norfloxacin (2). Tetracycline, erythromycin, levofloxacin, and cotrimoxazole showed one resistance each to H.pylori.A total of two strains were multi-drug resistant (MDR) . They showed resistance to metronidazole, amoxicillin, and norfloxacin.

Three metronidazole resistant strains were found in the patients with duodenal ulcer, gastric ulcer, and chronic gastritis.

DISCUSSION

In our study multiple tests were used for the detection of H.pylori in gastric biopsy specimens. The presence of H.pylori in gastric biopsie swas detected by urease test, H and E stain and culture In 28%, 14.5%, and 2.5% cases respectively. The overall positivity of rapid urease test correlates with another study, where out of 81 samples studied by rapid urease test 35 (43.21%) were positive. [14]. In another study done by Akanda et al., rapid urease test , and H and E staining detected H.pylori in 56.4% and 45.6% cases respectively.[[4]. The specificity of urease test is 81.7%. In another study the specificity of RUT was 60%.[15]. In the present study the sensitivity and specificity of Urease test in the demonstration of H.pylori is higher than culture. It is cheap easy to perform and available in most laboratories.

TABLE 1 :*SEX DISTRIBUTION WITH ENDOSCOPIC DIAGNOSIS*

SUBJECTS	MALE	FEMALE	TOTAL
Chronic gastritis	73(73%)	22(22%)	95(95%)
Duodenal ulcer	01(01%)	-	01(01%)
Gastric ulcer	02(02%)	02(02%)	04(04%)
TOTAL	76(76%)	24(24%)	100

In the present study,H.pylori was isolated in 3 out of 100 patients (2.5%), with a sensitivity of 13.3%.[Table 2]. Similar isolation rate has been reported in another study (4.2%).[15]. Studies from India have shown low rates of isolation.Ayyagari et al., reported 23.9% isolation rate[16]. In another study 8/92 (8.7%) samples were positive by culture with a sensitivity of 8.69%.[17]. In contrast the Indian studies reported a sensitivity which ranged from 1.09-63%.[16,18]. The sensitivity of our Culture is in accordance with these results . The low rate of isolation may be due to the fastidious nature of H. Pylori and a number of factors that are hard to control , such as patchy distribution of organisms on the gastric mucosa , loss of viability of organisms during transportation etc., All these factors together, result in low sensitivity and low negative predictive value [18]. Thus the need persists for a high H.pylori recovery rate from gastric biopsy specimens.

TABLE 2:DETECTION OF *Helicobacter pylori* IN GASTRIC BIOPSY SAMPLES BY DIFFERENT METHODS IN VARIOUS CONDITIONS (N=100)

TESTS	CHRONIC GASTRITIS		DUODENAL ULCER		GASTRIC ULCER ULCER		TOTAL	
	(95)	(%)	(01)	(%)	(04%)	(%)	(100)	(%)
	+	-	+	-	+	-	+	-
Urease	24(24.2)	71	1(100)	-	3(71.43)	1	28(28)	72
H&E	12(21.1)	83	1(100)	-	1(49.86)	3	15(14.5)	85
Culture	1(0.95)	94	1(100)	-	1(25.12)	2	3(2.5)	97.5

The presence of H.pylori in gastric biopsy was detected by H and E in 14.5%. In another study out of 65 samples processd for H and E , a total of 56 (86.15%) showed the changes associated with chronic gastritis.[17]. CDC recommends that histopathology[H and E] should be taken as gold standard. In comparison with H and E is better screening test when compared to culture.

Out of 28% cases detection by urease test 24.21% were chronic gastritis. So it can be used as one of the rapid test and can be used as bed side investigation. Gastritis can be having varied aetiology when urease test is used it will pinpoint at the diagnosis of H.pylori Infection since urease test has got 76.6% sensitivity. Thus urease test can be used for early diagnosis of dyspepsia. Culture has high specificity, which can be used as adjunct.

TABLE 3: SENSITIVITY AND SPECIFICITY OF DIFFERENT METHODS FOR IDENTIFICATION OF *Helicobacter pylori*

TEST	SENSITIVITY (%)	SPECIFICITY (%)	POSITIVE PREDICTIVE VALUE	NEGATIVE PREDICTIVE VALUE
Urease test	76.6	81.7	42.6	95.2
Culture	13.3	99.4	80.0	86.6

Patients were treated with H.pylori kit containing Pantoprazole 40 mg, clarithromycin 500 mg and amoxicillin 750 mg for 14 days then symptomatic improvement was assessed followed by maintenance dose of Pantoprozole 40 mg for 3 months. For gastric ulcer cases Pantoprazole 40 mg was put for 3 weeks then followed by antibiotic treatment after the symptomatic improvement.

Symptomatic profile of the patients before and after the treatment was assessed and there was endoscopic improvement after the treatment.Metronidazole resistant cases were put on quadruple treatment with proton pump inhibitors and three antibiotics.

Many H. Pylori strains show resistance to one or more antimicrobial agents in vitro and this may be the cause of eradication Failure.[19].Non-compliance of the patient and location of the bacterium wich is beneath the gastric epithelium , are involved in the treatment failure. Anitibiotic resistance varies widely by geographic location.Metronidazole is an important antimicrobial used in the treatment of H.pylori infection.[20]. Determination of antibiotic susceptibility, particularly to metronidazole is very essential.[21]. In our study, antibiotic susceptibility testing was carried out by disc diffusion method. It is a good alternative for determining antibiotic susceptibility of H. Pylori, particularly to metronidazole.[21,22]

TABLE 4: ANTIMICROBIAL PATTERN OF *H pylori* (N=03)

ANTIMICROBIAL AGENTS	RESISTANT
Metronidazole	**03**
Amoxicillin	**02**
Tetracycline	**01**
Erythromycin	**01**
Levofloxacin	**01**
Norfloxacin	**03**
Cotrimoxazole	**01**
Metronidazole +Amoxicillin	**02**
Metronidazole+Amoxicillin+Norfloxacin	**02**

In our study all the strains (03) were resistant to metroidazole. In another study H.pylori resistant rate was 77.9% to metronidazole, 32.8% to amoxicillin.[10].Metronidazole resistance was high in Lucknow,Chnnai, Hyderabad (68%, 88.2%, and 100% respectively and moderate in Delhi, (37.5%), and Chandigarh (38.2%). Ciprofloxacin And tetracycline resistance was the least ranging from

1.0-4 %.[10]. This is in accordance with our study.

The prevalence rate of metronidazole resistance is higher in developing countries [8]. This is due to general and extensive use of metronidazole in developing countries for other infectious problems such as protozoal, genital and dental infections [8,16]. In our study metronidazole has highest resistance rate.Primary resistance of H.pylori to metronidazole is consistent with earlier research conducted in several areas.[9].

There is lot of geographical variation in resistance pattern , indiscriminate use of drugs in different areas might be the cause of high metronidazole resistant H.pylori[23].

We found two of the strains resistant to amoxicillin.Frequent use of this drug for other infections like like respiratory conditions in our area may contribute to resistance.

In our zone, we got emerging resistance with norfloxacin (02). The reason for high resistance with norfloxacin is due to it's wide spread usage in the treatment of urinary tract infection.[23]

Although norfloxacin is not a drug of choice in the treatment of H.pylori infection,drug combination used in the H.pylori kit may be considered as an alternative in cases of resistance to first line drugs. [23].

one resistance was seen with other drugs like tetracycline, levofloxacin,cotrimoxazole, and erythromycin. The wide discrepancy in the antibiotic susceptibility pattern of H.pylori suggest the need A systematic approach to determine antibiogram of the strains before considering the drug regimens.

LIMITATIONS OF THE STUDY

The present study had certain limitations. The results of this study depicted low culture positives in controlled conditions existing. Further long term studies need to be conducted comparing the different cultivation methods and molecular techniques.

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