Rate of Isolation of *Helicobacter pylori* from Different Clinical Samples In Patients Suffering from Gastritis Attending Tertiary Care Hospital

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H.pylori is one of the most common infections in the world. It causes dyspepsia, chronic gastritis and gastric carcinoma. Spread of the infection from person to person is by oral-oral or faeco-oral route. The aim of the study is to determine the rate of isolation of H.pylori infection from different sites like gastric biopsy, gastric aspirate, saliva and faeces. Patients were selected based on the clinical findings like early satiation, epigastric pain, epigastric burning, bloating, belching and endoscopic findings. Four types of samples- Gastric biopsy, Gastric aspirate, Saliva and Faeces were collected and all the four samples were subjected to Direct Gram Stain, Rapid Urease Test and Culture. Culture was done by inoculating samples onto Skirrows media with cefsulodin and amphotericin B and incubated at 35ºC for 3-5 days under microaerophilic conditions. Identification of organism was done based on Gram stain of the colony, oxidase, catalase and urease test. Out of 96 clinically suspected cases of gastritis, H.pylori was isolated in 34(35.41%) patients from Gastric biopsy, 18 (18.75%) patients from Gastric aspirate, 8 (8.33%) patients from saliva and 4 (4.16%) patients from faeces. Among the 96 patients selected 59 were male participants and 37 were female participants with age between 20-60 years. In the present study, we found that H.pylori can be cultured from biopsy, gastric aspirate, saliva and faeces. There are several invasive and non invasive techniques to diagnose H.pylori, each having its own advantages and disadvantages. Non-invasive tests on saliva and faeces sample can play an important role in the diagnosis of H.pylori infection, but has the disadvantage of low sensitivity, whereas invasive tests like gastric biopsy and gastric aspirate are more sensitive.

Keywords: *H. pylori*; Gastritis; Gastric biopsy.

The presence of spiral organisms were first reported in the stomach of mammals over 120 year's ago[1]. Marshal tried to culture the bacteria by using methods to culture Campylobacter for 48 hours but was in vain[2]. The first successful culture occurred when the 35th biopsy culture was left in incubator for 5 days over the Easter holidays in April 1982. Marshal proved the association of the isolated bacteria as a causative agent of gastritis. As the bacteria were closely associated with Campylobacter it was named as *Campylobacter jejuni*[3]. Campylobacter became Helicobacter when it was found that the 16s ribosomal RNA did not have characteristic sequence found in Campylobacter[4].

H.pylori is a spiral shaped or curved microaerophilic Gram-negative motile bacteria equipped with 4-6 flagellae at one end. Gastric acid kills most bacteria but *H.pylori* has evolved some special features which allows them to live

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in acidic niche. Urea diffuses freely from plasma into gastric juice. The urease enzyme produced by *H.pylori* digests urea to produce carbon dioxide and ammonium hydroxide which neutralizes the gastric acid[5-9].

H.pylori causes dyspepsia, chronic gastritis and gastric carcinoma[10]. Spread of the infection from person to person is by oral-oral or faeco-oral route. It has been shown that *H.pylori* can survive up to 3-days in water[11].

The aim of the study is to determine the rate of isolation of *H.pylori* infection from different sites like gastric biopsy, gastric aspirate, saliva and faeces.

MATERIALS AND METHODS

Informed consent mentioning that data from the diagnosis would be used for research purpose was obtained from all the patients.

Inclusion Criteria

Patients were selected based on the clinical findings like early satiation, epigastric pain, epigastric burning, bloating, belching and endoscopic findings.

Based on endoscopic findings, the patients were divided into four groups:

Group-1: Normal findings

Group-2: Non-ulcerative with evidence of erythema, erosion, nodularity, atrophy, plaque or petechiae

Group-3: Ulcerative

Group-4: Both of group-2 and group-3

Patients pertaining to group 2, 3 and 4 were selected for the study

Exclusion Criteria

Patients who are on antibiotics, Antacids, pregnancy or history of gastrectomy were excluded from the study.

Methods

Patients were instructed by the gastroenterologist not to eat or drink for eight hours before endoscopy procedure. The patients received 10% xylocaine spray as local anaesthesia in the oropharyngeal region before the procedure. The endoscope and the biopsy forceps were cleaned by savlon and then sterilized by cidex for 10 minutes. Two biopsy specimens were collected from each patient, one for direct Gram stain and other for inoculation in culture media. Four types of samples were collected from each patient. The four samples are Gastric biopsy, Gastric aspirate, Saliva and Faeces. All the four samples were subjected to Direct Gram Stain, Rapid Urease Test and Culture[12, 13].

Direct gram stain showed S-shaped bacilli 2.5 to 4 mm long and 0.5 to 1 mm thick. All the specimens were inoculated onto Skirrows media with cefsulodin and amphotericin B (Dent's selective media) and incubated at 35ºC for 3-5 days under microaerophilic conditions.

The colonies were discrete and translucent. Identification was done based on Gram stain of the colony, positive oxidase, catalase and urease test[12].

RESULTS

Based on clinical findings, a total number of 147 endoscopies were done in a period of 6 months, of which 96 (65.3%) patients were falling under group 2, 3 or 4. Among the 96 patients selected 59 were male participants and 37 were female participants with age between 20-60 years. *H.pylori* was isolated in 34 patients from Gastric biopsy, 18 patients from Gastric aspirate, 8 patients from saliva and 4 patients from Faeces. The isolation of *H.pylori* from Gastric biopsy was

Table 1. Rate of isolation of *H.pylori* from different clinical samples from patients suffering from gastritis is shown in table below:

Samples	No. of Isolation of *H.pylori*(n = 34)	(%)
Only Gastric biopsy positive	34	35.41
Gastric biopsy* and Gastric aspirate positive	18	18.75%
Gastric biopsy and Saliva positive	8	8.33%
Gastric biopsy and faeces positive	4	4.16%

* Gastric biopsy is taken as gold standard specimen for *H.pylori* isolation

Table 2. Demographic and Clinical Characteristics
of Patients Included In the study

Variables	Total number of patients n=96	H.pylori positive (n=34)	%
Sex			
Male	59	21	35.59
Female	37	13	22.03
Age			
20-30 years	28	11	39.28
30-40 years	39	14	35.89
40-50 years	21	8	38.09
50-60 years	8	1	12.5
Endoscopic findings			
Gastritis	58	19	32.75
Gastric ulcer	38	15	39.4
Gastric carcinoma	Nil	Nil	Nil

35.41%, from gastric aspirate was 18.75%, from saliva 8.33% and from faeces 4.16%.

DISCUSSION

In the above study, we have observed that the sensitivity of gastric biopsy was much more compared to gastric aspirate, saliva and faeces respectively.

The study by Oshowo A *et al* reported 17% isolation of *H.pylori* from gastric juice[14], 55% from gastric biopsy, which showed that the isolation of *H.pylori* from biopsy was high when compared to the results of present study, whereas the results were almost similar for gastric juice. Contrary to our work, Allaker RP *et al* reported 25% isolation of *H.pylori* from faeces in gastric biopsy positive patients, which was high[11].

As per Young KA *et al* report 50% positive results from Gastric biopsy and 37% from Gastric juice[15] and according to Momtaz H 10.72% isolates were from saliva, 71.67% from faeces and 77.88% from gastric biopsies[16] . These results were also in increasing trend compared to the present study.

The observations made by Matsukura N *et al*[17] showed the isolation of *H.pylori* in gastric aspirate to be 38% and biopsy 40%[17]. Uribe R *et al*[18] isolated 94.1% of *H.pylori* from gastric aspirate by PCR, which is more sensitive than culture.

In our study, the prevalence of *H.pylori* was more in males (35.9%) when compared to females (22.03%). The rate of *H.pylori* infection was more in males when compared to females. Similar results were shown by Khan AR *et al*[19] where the rate of infection in males was 69% and females was 31%. He also showed that there was no significant difference between different age groups. Kanbay *et al*[20] showed that the rate of infection increased along with the age and women were infected more commonly than men. Whereas our study showed that the rate of infection is low between 50-60 years of age group and men are more commonly infected than women.

CONCLUSION

The *H.pylori* is one of the most common infections in the world. It is present in 70-90% of the population in developing countries and 25–50% in developed countries[21].

In the present study, we found that *H.pylori* can be cultured from biopsy, gastric aspirate, saliva and faeces. *Helicobacter pylori* were less reliably cultured from stool than from saliva. There are several invasive and non invasive techniques to diagnose *H.pylori*, each having its own advantages and disadvantages. Non-invasive tests on saliva and faeces sample can play an important role in the diagnosis of *H.pylori* infection, but has the

disadvantage of low sensitivity, whereas invasive tests like gastric biopsy and gastric aspirate are more sensitive.

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