ORIGINAL ARTICLE

HELICOBACTER PYLORI DETECTION IN CHRONIC GASTRITIS: A COMPARISON OF STAINING METHODS

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Background: Helicobacter pylori is an important cause of chronic gastritis**,** gastric ulceration and gastric malignancies as gastric carcinoma and MALT lymphoma. Its definitive diagnosis is based on histopathology. Routine H & E stain is not very effective in its detection, immune-stains and fluorescent stains are costly. Need for simple cheap and sensitive stain has always been a topic of hot debate and extensive research. **Method:** paraffin embedded blocks of all adult patients diagnosed as chronic gastritis/gastric ulceration with no accompanying gastric pathology as hypertrophic gastropathys, and neoplasias were taken into study. Three sections of 4 micron were cut and stained with routine H & E, Giemsa, and Cresyl fast violet. **Results:** Total number of patients was 50. Out of these 37 (74%) were males and 13 (26%) were females. Mean age of the patients was 50.4 years. Thirty-four percent (34%) were positive in normal H & E stain, 68% were positive in Giemsa and 76% were positive in Cresyl fast violet. **Conclusion:** Cresyl fast violet is a good stain for diagnosis of H. pylori gastritis.

Keywords: H pylori, chronic gastritis, H pylori staining methods

INTRODUCTION

Chronic gastritis and gastric ulceration are common problems worldwide and also in Pakistan.[1] Previously many etiologic agents have been implicated in the causation of this disease such as smoking[2], non-steroidal anti inflammatory drugs (NSAIDs)[3], spicy foods and an influence of personality status.[4] But now it has been proved that in addition to these *Helicobacter pylori*, a bacterial infective agent, is the most common cause of this disease.[5] It is not only claimed to be the primary cause of gastric ulcerations but also it acts as a synergist to produce gastritis and gastric ulceration with smoking, non steroidal anti inflammatory drugs, and other predisposing conditions.[6] Most important fact about the infection with this organism is that not only it causes gastritis and gastric ulceration but also leads to malignancies such as adenocarcinoma and MALT lymphoma of stomach.[7,8]

The diagnosis of *H. Pylori* gastritis can be made through many laboratory tests. The techniques are divided into two groups the invasive and non-invasive tests**.**[9] The invasive tests include, stomach biopsy, culture and CLO test. Non-invasive tests include urea breath test and serological test for measurement of antibodies against *H. pylori*.

The gold standard for the diagnosis is detection of *H. pylori* in biopsy material. The organism can easily be seen in a histological section of gastric mucosal biopsy stained with Giemsa, Cresyl fast violet, Acridine orange and routine H & E stains. Immuno-stain is the gold standard among stains.[10]

The present study aims at confirming the relative efficacy of three cheap stains, i.e., Giemsa, Cresyl fast violet, and normal H & E stain.

MATERIAL AND METHODS

The study was conducted at the Department of Pathology, Ayub Medical College Abbottabad in collaboration with the Department of Pathology Postgraduate Medical Institute Lahore. Sample collection was done from 1st of November 2009 to 25th of April 2010.

The study consisted of 50 histopathologically diagnosed cases of gastric ulceration and chronic gastritis. The sampling technique was convenience (Non probability) sampling. This was a comparative study between three stains, Giemsa, Cresyl fast violet and normal H & E stain.

Routinely processed, paraffin embedded tissue blocks diagnosed as gastritis/gastric ulceration were selected. Three sections of 4 μ thickness were cut from each block and mounted on three slides one each on a slide. One slide was stained by routine H & E stain; the other two by Giemsa and Cresyl Fast Violet stains respectively. Slides were mounted on by cover slips with DPX. Thorough microscopic examination was done using Olympus binocular microscope model CH - 3 series.

In positive cases, the bacteria appear as light bluish rods in H & E slides with varying sizes (3–6 μ) on the luminal surface of mucosal cells. In Giemsa stain *H. pylori* appears dark blue in a light blue background. In Cresyl violet stain it appears as dark purplish in a light purple background[11] Presence of *H. pylori* in any of the slides was taken as a positive for infection. All data were recorded on a Proforma and analysed using SPSS-17 for frequencies, ratios, percentages, and Mean±SD. Fisher's exact test was applied to see the significance, $p<0.05$ was taken as significant.

RESULTS

Out of the 50 patients 37 (74%) were males and 13 (26%) were females. Twenty-eight percent were from 20–40 years, 48% were from 41–60 years and 24% were >60 years of age (Table-1). Maximum positivity of *H. pylori* was with Cresyl Fast Violet stain, i.e., 38 cases (76%), followed by Giemsa stain detecting 34 cases (68%). Routine H & E could only detect 17 cases (34%) (Table-2). As Cresyl Fast Violet shows 76% positivity which is very close to international studies showing 80% correlation of *H. pylori* with gastritis and gastric ulceration it can be taken as gold standard in this study. Using 2×2 table Giemsa and routine H & E stain could be compared with Cresyl Fast Violet.

Cross-tabulating Giemsa with Cresyl Fast Violet in a 2×2 table shows a sensitivity of 84.47% and specificity of 100% with a positive predictive value of 100% and a negative predictive value of 75%. The *p*-value calculated by Fisher's exact test was <0.05, and was significant.

With H & E stain sensitivity was 44.7%, specificity was 100% with a positive predictive value of 100% and a negative predictive value of 36.36%, (p<0.05).

Table-1: Age group (n=50)

Age Group (Years)	Frequency	Percentage
20–40	14	28.0
41–60	24	48.0
>60	12	24.0

Table-3: Stain positivity

Stains	Positivity	Percentage
Cresyl fast violet	38	76
Giemsa	34	68
H & E	17	34

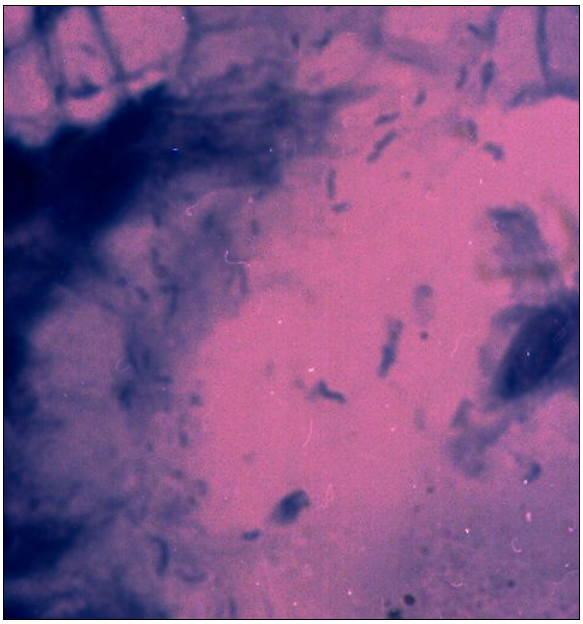

Figure-1: Cresyl Fast Violet Stain

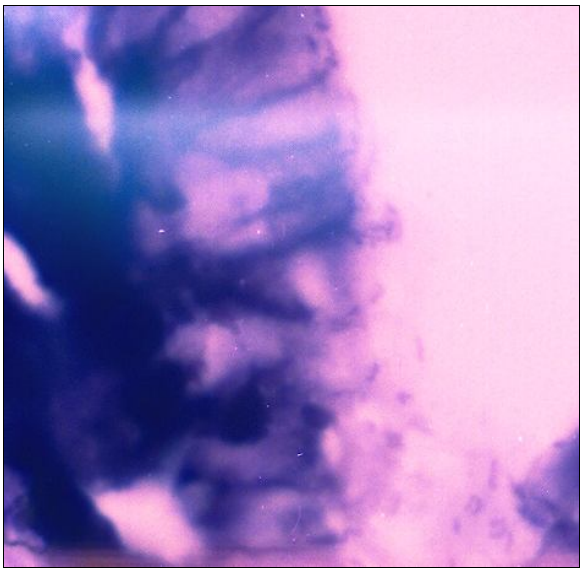

Figure-2: Giemsa Stain

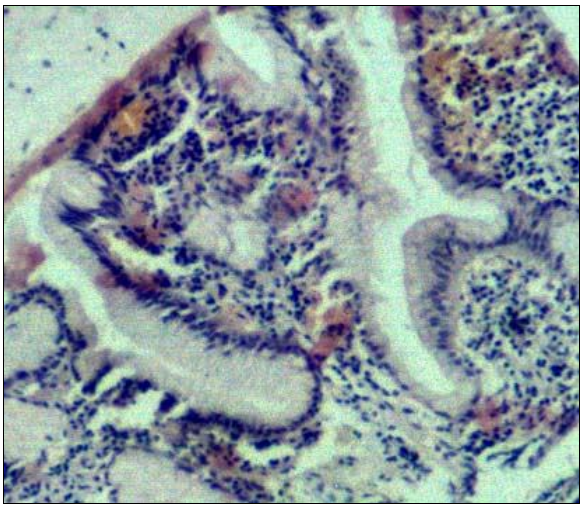

Figure-3: H & E Stain

DISCUSSION

Gastric ulceration is a common problem in Pakistan[1] and in almost all developing countries[12]. Majority of these cases have a strong association with *H. pylori* infection. *Helicobacter pylori* infection is not only associated with high incidence of gastric ulceration but also has a positive correlation with gastric MALT lymphoma and some gastric carcinoma cases.[13,14]

Hence it is imperative to identify this infection at an early stage and to treat this condition at an earliest stage to avoid lethal complications. At the same time it is important to know the frequency of this infection in our society for its early eradication.

Results of this study show a significant high positivity of *H. pylori* infection (78%) in gastric ulcers which is very close to the international total, i.e., (70–80%), as narrated by Cotran *et al*.[15] Walsh *et*

al[16] in their study documented an 80% correlation of gastric ulcer with *H. pylori* but stress more on socioeconomic status of the patients while in our study socioeconomic groups were not taken into consideration. Mitchell[17] *et al* have documented a strong correlation of *H. pylori* with gastric ulcers but have stressed more on age of acquiring infection and have not given exact figures. Porth[18] *et al* has stated that *H. pylori* has a frequency of around 84.4% in people having gastritis and gastric ulceration. Abbas[19] *et al* say that in 70–80% of the cases, symptoms rapidly abate if given eradication therapy for *H. pylori*. We therefore assume 80% of the cases to be caused by *H. Pylori* as is shown by other studies, then detection by Cresyl fast violet is very near to it approximately detecting all the cases, followed by Giemsa stain.

In our study Cresyl fast violet stain proved best with 78% positivity, followed by Giemsa stain (68%) and H & E stain (34%) respectively (Figure-1,2,3).

Results of the last stain are in accordance with Rotimi[19] being less sensitive.

Rotimi[20] *et al* has documented that after the gold standard immuno-stain, the Giemsa is the best and most sensitive stain for *H. pylori*, but stains he compared, did not contain Cresyl fast violet. Haqqani[21] *et al* has claimed Acridine orange, a fluorescent stain[22], to have an equal sensitivity as immuno-stain and in a set of experiment has proved that a number of cases positive with acridine orange were positive with the gold standard immuno-stain. Acridine orange was not included in this study and a new study is needed to evaluate this stain.

CONCLUSION

Overall this study reveals that there is a significant positivity of Cresyl fast violet and this stain is very cheap and one step procedure not time consuming therefore it can be used for detection of *H. pylori* in routine histopathology.

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