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Histological Detection of Helicobacter Pylori: Comparison of Giemsa and Immunohistochemistry Techniques in Gastric Adenocarcinoma Specimens

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Abstract

Background: The various histological methods of identification of Helicobacter pylori have different sensitivity and specificity with varying strengths and weaknesses in terms of cost and convenience. *Aim:* The aim of this study is to compare the efficacy of Giemsa stain with Immunohistochemistry in diagnosing Helicobacter pylori in paraffin embedded tissue sections. *Materials and Methods:* This is a retrospective study done in a tertiary care centre for a period of 3 years. Multiple bits were taken from the lesion and the adjacent areas in random sample of 50 gastrectomy specimens and processed routinely for paraffin embedding. Sections were stained for Giemsa and immunohistochemistry apart from the routine Haematoxylin and eosin stain. *Statistical Analysis:* The data were entered and the statistical comparison between the two tests were done using SPSS software version 21. *Results:* Out of 50 cases of adenocarcinoma stomach, 39(78%) were males and 11(22%) were females. Out of 50 cases H.pylori was detected in 21 cases using Giemsa stain with 42% of positivity (Table.1). Out of 50 cases, Immunohistochemistry detected H.pylori in 25 cases (4 cases more than Giemsa) with a positivity rate of 50%. *Conclusion:* Sensitivity of Immunohistochemistry is more when compared with Giemsa special staining. Immunohistochemistry can be used to detect coccoid forms of Helicobacter pylori especially in screening for the presence of Helicobacter pylori in high risk patients and also in patients who had been treated with proton pump inhibitors and to confirm eradication following antibiotics against Helicobacter pylori.

Keywords: Helicobacter Pylori; Giemsa; Immunohistochemistry; Gastric Adenocarcinoma; Gastric Biopsy.

Introduction

Helicobacter pylori is the most prevalent human infection of the globe affecting nearly half of the world's population [1]. It was first identified in 1984 by Marshall and Warren from culture of gastric biopsy [2]. Many studies [3–5] have found an association between Helicobacter pylori and gastric carcinoma and in 1994, the World Health Organisation identified Helicobacter pylori as a carcinogen [6]. So in view of its pathogenic significance, it is essential to identify this spiral organism in suspected cases to initiate appropriate eradication protocols. The histological identification and demonstration of the bacteria in the biopsy specimens is the most widely practiced method worldwide which includes several special staining techniques and immunohistochemical antibody stains. These staining methods have different sensitivity and specificity with varying strengths and weaknesses in terms of cost and convenience [7–10]. Therefore there is always no consensus method of identifying helicobacter pylori in histology. Further the technological capabilities of the laboratory, availability of expert gastrointestinal pathologist also determines the diagnosis of this pathogen. Thus in this study we aimed to compare the identification of the Helicobacter pylori in gastric adenocarcinoma specimens using Giemsa stain which is cost effective, technically easy to perform stain with that of immunohistochemistry, considered as a Gold standard test.

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Aims and Objectives

The aim of this study is to find out the incidence of Helicobacter pylori in gastrectomy specimens using Giemsa stain and Immunohistochemistry (IHC) using (polyclonal antibody to Helicobacter pylori antigen) in patients who were operated for gastric adenocarcinoma. An attempt was made to compare the efficacy of Immunohistochemistry over Giemsa stain in diagnosing Helicobacter pylori in paraffin embedded tissue sections.

Materials and Methods

Study Design

This is a retrospective study carried out in a tertiary care centre for a period of three years. A total of 60 gastrectomy specimens with adenocarcinoma were received during the study period and out of this, a random sample of 50 gastrectomy specimens were taken for this study. For all the 50 cases, details of age, sex were recorded. Depending upon the site of growth, the stomach was opened through the greater or the lesser curvature. The gross appearance and three dimensional measurement were taken. Multiple bits from the lesion and the adjacent areas were taken and processed routinely for paraffin embedding. 4 micron sections were taken and stained by H&E. Sections were further stained by Giemsa stain.Sections were also mounted on chrome alum coated slides.Giemsa stained sections were used for recording the presence of Helicobacter pylori. Sections mounted on chrome alum coated slides were used for immunohistochemistry.

Statistical Analysis

The data were entered and analysed using SPSS software version 21. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio for the two tests were analysed and compared.

Results

Out of 50 cases of adenocarcinoma stomach, 39(78%) were males and 11(22%) were females. Out of 50 cases H.pylori was detected in 21 cases using Giemsa stain with 42% of positivity (Table.1). Out of 50 cases, Immunohistochemistry detected H.pylori in 25 cases (4 cases more thanGiemsa) with a positivity rate of 50% (Table.2). Comparison of Giemsa and Immunohistochemistry in detecting Helicobacter pylori is shown in Table 3 and Table.4.

Table. 1: Detection of H.pylori with GIEMSA STAIN:

GIEMSA	No. of patients	Percent
Negative	29	58.0
Positive	21	42.0
Total	50	100.0

Table 2: Detection of H.pylori with polyclonal antibodies against H.pylori antigen by Immonohistochemistry

IHC	No. of patients	Percent
Negative	25	50.0
Positive	25	50.0
Total	50	100.0

Table 3: Comparison of IHC and GIEMSA

	Helicobacter pylori status	GIEMSA	
		Positive	Negative
Immunohistochemsitry	Positive	21	4
	Negative	0	25

Table 4: Statistical comparison of Giemsa with the gold standard Immunohistochemistry

	Estimated value	Confidence Interval
Sensitivity	84%	63-94%
Specificity	100%	83-100%
Positive predictive value	100%	80-100%
Negative predictive value	86%	72-94%
Positive likelihood ratio	∞	∞
Negative likelihood ratio	0.16	0.07 to 0.39

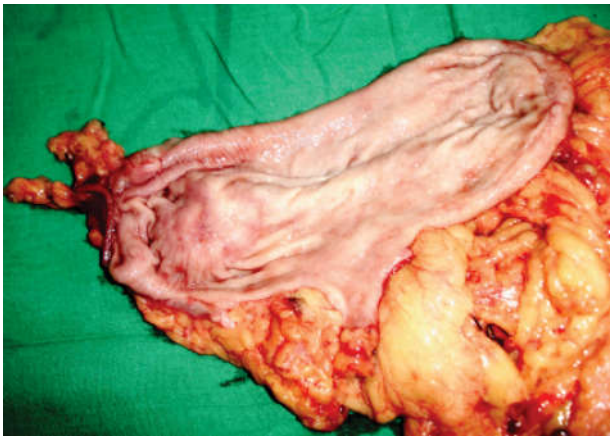

Fig. 1: Picture showing a specimen of Gastric adenocarcinoma

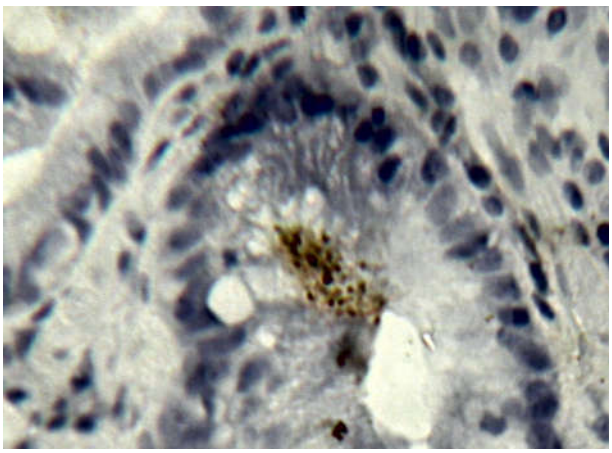

Fig. 2: Picture showing Immunohistochemistry positivity for Helicobacter pylori

Discussion

When compared with Giemsa stain, Immunohistochemistry has detected 4 cases more (25/50). The giemsa stain has a sensitivity of 84% and specificity of 100% and a positive predictive value of 100% and a negative predictive value of 86% and negative likelihood ratio of 0.16. The k agreement status(k=0.84 and p=<0.001) is correlating between the two histological diagnostic methods – Giemsa and the Immunohistochemistry.

Much debate have been made on the diagnosis of Helicobacter pylori between the non invasive tests like Urea breath test, rapid urease test and the invasive tests like histology and culture. Studies [8,11] have revealed little difference in the specificities between these tests, but histology was more sensitive than the rest of the tests. Though the sensitivity of histology is higher it is much affected by sampling and observer bias. The topographical localization of the bacteria plays a very significant role in the biopsy specimens accounting for the sampling errors [12,13]. Though sampling from the pylorus can reveal the colonization in most cases, additional sampling from the antrum and body can increase the chance of detection [14]. Interobserver variation is very high and the chance of detection is very variable if the biopsy samples are examined in routine Hematoxylin and eosin stain only even after careful examinations [15,16]. As Giemsa staining is very simple to use, cost effective and provides consistency in the results, it is widely used in most diagnostic laboratories and hospitals [17]. Warthin starry silver stain is not widely used because of its expensiveness and inconsistency[18]. Genta stain has the advantage of combining silver stain, hematoxylin and eosin and Alcian blue [19], but it involves a complex staining techniques and time consuming and hence was not routinely practiced.

Immunohistochemistry is regarded as the gold standard method for identifying the Helicobacter pylori organism because it is highly sensitive and reliable technique. Its advantage over other histological methods are identification of the atypical coccoid form of the organism in partially treated patients and differentiating it from other bacilli with similar morphology and cell debris [20].

Hence in routine practices, it can be suggested that Giemsa stain can be included in all gastric biopsies in addition to the routine Haematoxylin and eosin stain. Immunohistochemistry can be added in special situations when there is a strong evidence of inflammation in the biopsy but the bacilli is not demonstrable in Haematoxylin and eosin or Giemsa staining, or to ensure the successfulness of the eradication treatment in special cases and to differentiate the Helicobacter pylori from other morphologically similar bacilli [21].

Conclusion

Sensitivity of Immunohistochemistry is more when compared with Giemsa special staining. Immunohistochemistry can be used to detect coccoid forms of Helicobacter pylori especially in screening for the presence of Helicobacter pylori in high risk patients and also in patients who had been treated with proton pump inhibitors and to confirm eradication following antibiotics against H. pylori.

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