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RESEARCH ARTICLE

Reliability of histological staining methods in the detection of Helicobacter pylori: comparison of three staining methods.

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Abstract

Objective: A reliable diagnosis of Helicobacter pylori (H. pylori) is important in clinical practice and research. The aim of this study was to evaluate some quality parameters of two histological staining methods routinely used for the histological identification of H. pylori microorganism.

Materials and methods: Hematoxylin and Eosin (H&E), Modified Giemsa and Gimenez stains were used for the staining of histological sections prepared from 45 gastric biopsies taken from adult patient attended at Khartoum teaching hospital for gastrointestinal endoscopy for evaluation of gastroduodenal diseases. Modified Giemsa stain results was used as a reference for the calculations of sensitivity, accuracy ratio and Negative predictive value (NPV) for H&E stain compared to Gimenez stains.

Results: Out of 45 processed gastric biopsies**,** H.pylori was identified in 12(27%) sections stained with Modified Giemsa stain, 9(20%) with Gimenez stains and 6(13%) with H&E stain. In reference to Modified Giemsa stain results, the sensitivity, and accuracy ratio and NPV of the Gimenez stain were (75%, 93.3and 91.7) compared to (50 %, 86.6% and 84.6%) for H&E stain.

Conclusion: Our study concluded that the performance of Gimenez stain in detection of H.pylori in tissue section was quite better than H&E. Although no statistical difference but Gimenez stain appears to be preferred stain for H.pylori detection based on it is good sensitivity, excellent accuracy and negative predictive value.

INTRODUCTION

Infection by Helicobacter pylori has been established as the major cause of chronic gastritis, and important in the pathogenesis of the other gastroduodenal diseases such as peptic ulceration, gastric lymphoma and gastric cancer.[1] therefor, the accurate detection of the organism is essential for correct patient management and it is particularly important to confirm eradication of bacteria following treatment. [2] various techniques are used for this purpose, including, serology, culture, rapid urase test, C-urea breath test and histology.[3]

As the standard method to diagnose H. pylori infection, histological examination provides critical information related to the mucosa (e.g., presence and severity of inflammation, intestinal metaplasia, glandular atrophy, dysplasia, and neoplasia). Several studies have recommended that both antrum and corpus biopsies be collected.[4]

The histological identification of H pylori infection is now a widely used means of diagnosis. To achieve this, several staining methods are in use, including modified Giemsa, Warthin-Starry, Gimenez, Genta, and

immunohistochemistry H pylori antibody stains. Immunohistochemistry is the agreed "gold standard" for histology, being a highly sensitive and specific staining method.[5]

Various special stains have been devised to detect H.pylori in these histological sections but their specificity and sensitivity vary greatly. The haematoxylin and eosin stain, the most frequently used stain in histology, has been found to be the most unreliable.[6] The silver stain, though found to be more superior, is quite complicated to carry out and the granular appearance it gives the organisms may be confused with silver precipitate.[7] Modified Giemsa stain described by Gray et al (1986) [8] has been favored by many researchers because of its easiness to perform and availability in most histopathology laboratories. [9]

Materials & methods

This was laboratory based study of two histological stains used in detection of H.pylori compared in reference to modified Giemsa stain. A total of 45 gastric biopsies taken from adult patient attended at Khartoum Teaching Hospital during the period from March to July 2011 for the evaluation of gastroduodenal disease by gastrointestinal endoscopy. The collected specimens were fixed in 10% formal saline for 24 hours and then dehydrated in increasing concentrations of isopropyl alcohol followed by clearing of alcohol by xylene before impregnating in paraffin wax. The specimens were subsequently embedded in paraffin wax in cassettes to facilitate tissue sectioning. Then standard staining procedure was performed on (5µm) sections from each specimen block and examined for the detection of gastritis and H.pylori.

Staining methods

All sections were stained with Modified Giemsa, H&E and Gimenez stains method.

Hematoxylin and Eosin

The section were stained in Hematoxylin (Mayer's Hematoxylin) for five to ten minutes and washed in running tap water for five to ten minutes. The sections were then stained with one per cent aqueous eosin for five minutes, the slides were washed with tap water, dehydrate in two changes of absolute alcohol for two minutes in each and clear in xylene for one minute.

Modified Giemsa stain

The fixative is not critical but b5 or Zenker is preferred thin (3Mm) paraffin section. Which was consist of Giemsa stain powder 4 g, glycerol 250ml,methanol 250ml, acetate buffer, distilled water at PH 6.8 were Dissolve powder in glycerol at 60C with regular shaking the added methanol, mix and allowed to stand for 7days filter before use.

Gimenez stain

The fixative is not critical with (3 or 4Mm) paraffin section. Which is consisting of buffer solution (phosphate buffer at PH 7.5 or o.1 M), stock carbol fuchsine and malachite green were filtered before use.

Mounting of the sections

Cover slip of 24 X 50 millimeter size was used to protect the sections. The mounting media used was Canada balsam in xylene neutral Thai (BDH Chemical LTD, England). It was applied as a streak between the slide and the cover slip. The prepared slides were left for 24 to 48 hours at room temperature to dry.

Microscopic examination of the histological sections

Histological sections stained with Modified Giemsa, Gimenez and H&E were examined under oil immersion objective (1,000×) by a pathologist for the classification of gastritis and by researchers for H. pylori infection.

Statistical analysis

Sensitivity, accuracy ratio and negative predictive value were calculated for comparative analysis. The P value less than or equal to 0.05 was considered significant for Chi-squire test ($\chi 2$).

Results

A total of 45 biopsy specimens taken during endoscopic examination from patient with age mean of 45 years fit the criteria of this study. There were 58% (26/45) females and 42% (19/45) in this study with age range (17-83) years. The distribution of age among the study population shown in figure 1

The histological diagnosis showed that, 68% diagnosed as normal histological finding, 15.4% presenting chronic gastritis, 6.6% presenting a final diagnosis of peptic ulcer, 4.4% diagnosed as gastrointestinal stromal tumor and 4.4% diagnosed as carcinoma as shown in figure 2.

Table 1 shows that H.pylori detected in histological sections stained by Modified Giemsa stain, Gimenez stain and H&E were 27%(12/45), 20% (9/45) and 13% (6/45) respectively, with no statistical difference (P=0.286). No cases were thought to be definitely positive on Modified Giemsa and found to be negative by Gimenez or H&E stains. Table 2 shows the using of Modified Giemsa stain results as the reference, the sensitivity, accuracy ratio and negative predictive values of Gimenez and H&E stains for the detection of H.pylori in histological sections were (75%, 93.3and 91.7) and (50 %, 86.6% and 84.6%) respectively.

Discussion

HP infection is the most common cause of chronic active gastritis. Several methods and techniques, including culture, rapid urease test, H&E stains, special stains, and immunohistochemistry stains, are available to pathologists and clinicians for the detection of HP in gastric biopsy specimens.[10]

The sensitivity and specificity of histological stain for detection of H. pylori depends not only on the number and site of the biopsies but also, including the staining technique and experience of the pathologist. [11] However, A few studies have investigated the sensitivity and specificity of the different staining methods. Although the various methods have their strengths and weaknesses, none has been shown to be superior to others in terms of cost, convenience, and sensitivity. [3]

Our study curried out to evaluate the sensitivity, accuracy and negative predictive value of histological staining methods (Gimenez and H&E) used for the detection of H.pylori in gastric biopsies. The study results demonstrate in significance difference in compared stains performance. This finding is similar to the results reported by Himani B.P et al on a comparative study of different staining methods.[12]

High number of detected H.pylori organisms was detected by Giemsa stain in comparison with the other stains (table1). This finding confirm that Giemsa stain is the more sensitive and providing definitive diagnosis that have important consequences for patient management which makes it a justifiable means of diagnosing H.pylori infection.[3] So, based on this findings the Giemsa stain detection result was considered a standard reference to measure the performance of the other stains (Gimenez and H&E).

The sensitivity, accuracy ratio and negative predictive values were calculated for the other two stains. H&E stain presented low sensitivity in comparison with Gimenez stain (table 2), similarly Himani B.P et al reported that the H. pylori can be easily identified by H&E stain in most cases, but nevertheless the sensitivity is low, particularly when the numbers of bacteria are less.[12] Also, it is reported that H&E staining is usually adequate and Giemsa stain seems to have advantage over other stains because of its simplicity and consistency. Moreover, previous a study evaluated the reliability of H. pylori identification on H&E-stained gastric biopsy specimens' results in very poor sensitivity (66%) and suboptimal specificity (88%). Furthermore, H&E stain can directly identify H. pylori in a high magnification field and evaluate the degree of inflammation. However, when a low density of H. pylori and atrophic mucosal change are combined, it becomes difficult to see the organism. [13]

Conclusion

In conclusion, we compared the performance of three histological stains. By using the result of modified Giemsa stain technique as reference, Gimenez stain demonstrates highly sensitivity method for identifying H.pylori in gastric biopsy specimens. It is more sensitive than traditional H&E stain and considerably easier to prepare, use and interpret.

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Table 1: Numbers of detected H.pylori in stained tissue sections

Staining method	Positive		Negative		Total	$\chi 2$	P
	No.	%	No.	%			
Giemsa	12	27%	33	73%	45		
Gimenez	9	20%	36	80%	45	2.5	0.286
Hematoxylin and eosin	6	13%	39	87%	45		

Table 2: Sensitivity, accuracy ratio and NPP of compared stains

Staining method	Sensitivity	Accuracy	NPV
Gimenez	75.0%	93.3%	91.7%
Hematoxylin and eosin	50.0%	86.7%	84.6%

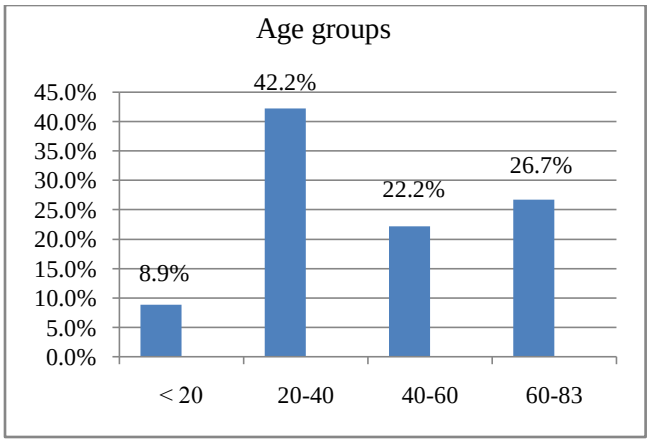

Figure (1) Age distribution among study population

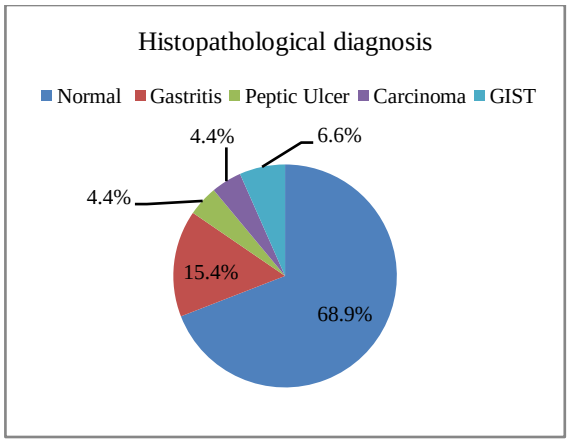

Figure (2) Distribution of Histological diagnosis