Diagnosis for H-Pylori by Various Staining Methods in Gastric Biopsy Specimens

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ABSTRACT
Objective: To run a comparison between specificity, sensitivity, PPV (positive predictive value) (PPV) & NPV (negative predictive value) of the three varied H. pylori detection methods used in the study.

Materials and Methods: It is a retrospective descriptive study that was carried out at Histopathology wing of Pathology Department, Sahiwal Medical College, Sahiwal. It comprised of entire cases of gastritis which were histopathologically proven by using Hematoxylin and Eosin, Giemsa and Immunohistochemical stains on biopsies taken by endoscopy and were sent from Gastroenterology Department Govt. Haji Abdul Qayyum Teaching Hospital, Sahiwal from January 2020 to the month of December 2020. SPSS version 20 was employed to evaluate all the received data.

Results: Amongst 95 samples, IHC (Immunohistochemistry) was ranked highest, displaying 100% specificity, 100% positive predictive value and 94.74% sensitivity. The H&E stain followed IHC, displaying 100% and 94.44% specificity and sensitivity respectively. The Giemsa stain was the runner up, displaying 95.65% specificity and 94.12% sensitivity.

Conclusions: Histologically, H. pylori infection was associated more with chronic active gastritis as compared with chronic gastritis. The three dissimilar staining methods achieved sensitivity, specificity, positive predictive value and negative predictive values equally well. IHC is a precise method for detection of H.Pylori in gastric biopsies with high sensitivity and specificity. It is suggested to use more than one staining method where H.pylori level is low, it will minimize false-negative rate.

Keywords: Helicobacter pylori, Diagnosis, Staining, IHC, H & E, Giemsa

INTRODUCTION
Gastric infection caused by the spiral, gram-negative H. pylori (Helicobacter pylori) is established to have a connection with a host of upper gastrointestinal diseases. In fact, H. pylori is held responsible for half of the infections caused in the world, being labelled by the WHO as a “definite biological carcinogen” in 1994. H. pylori has a definite role in about 80% of carcinomas of stomach as well as 92% of low-grade gastric MALT (mucosa-associated lymphoid tissue). Presently, a combination of non-invasive and invasive diagnostic tools have been established to be able to better diagnose H. pylori. An array of histochemical stains is employed to better recognize the samples generated from gastric biopsies and resections. They include altered Giemsa, Gimenez, cresyl violet, acridine orange, Ziehl-Nelsen (ZN) stain, Genta, half Gram and H. pylori stain that is silver and modified Genta. The use of different histochemical stains leads to enhanced visualization of the system in contrast to the hematoxylin and eosin (H&E) stain. Nonetheless, numerous studies have indicated that staining with immunohistochemistry (IHC) with precise Polyclonal H. pylori antibodies yields the sensitivity with highest specificity and improved variation of results between different observers in contrast to the use of different histochemical stains along with other diagnostic tests.

This study focused on carrying out a histological analysis of the gastric biopsies with the correlation to the evaluation of infection by H. pylori. We elaborate on the sensitivity, specificity, PPV, NPV of Giemsa, H & E as well as IHC in H. pylori positive infection.

MATERIAL AND METHODS
This was a retrospective descriptive investigation carried out at histopathology wing of Pathology Department, Sahiwal Medical College, Sahiwal. It comprised of entire cases of gastritis which were histopathologically proven by using Hematoxylin and eosin, Giemsa and Immunohistochemical stains on biopsies taken by endoscopy sent from the Department of Gastroenterology, Govt. Haji Abdul Qayyum Teaching Hospital, Sahiwal from January 2020 to the month of December 2020. SPSS version 20 was used to analyze all the data received.

RESULTS
Total 95 gastritis cases were registered in this study and comparison was done using three distinct stains, H&E, Giemsa and IHC between H.Pylori positive & H.Pylori negative endoscopic biopsies (Tables 1, 2 and 3). Sensitivity, specificity, PPV and NPV values were calculated. IHC (Immunohistochemistry) was ranked highest, displaying 100% specificity, 100% positive predictive value and 94.74% sensitivity. The H&E stain followed IHC, displaying 100% and 94.44% specificity and sensitivity respectively. The Giemsa stain was the runner up, displaying 95.65% specificity and 94.12% sensitivity (Table 4).

Table 01: H & E stain of H.Pylori on Endoscopic Biopsies

<table>
<thead>
<tr>
<th>Stain</th>
<th>H.pylori +ve</th>
<th>H.pylori -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>H &amp; E +ve</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td>H &amp; E -ve</td>
<td>04</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 02: Giemsa stain of H.Pylori on Endoscopic Biopsies

<table>
<thead>
<tr>
<th>Stain</th>
<th>H.pylori +ve</th>
<th>H.pylori -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giemsa +ve</td>
<td>66</td>
<td>01</td>
</tr>
<tr>
<td>Giemsa -ve</td>
<td>04</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 03: Results of IHC stain of H.Pylori on Endoscopic Biopsies

<table>
<thead>
<tr>
<th>Stain</th>
<th>H.pylori +ve</th>
<th>H.pylori -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC +ve</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>IHC -ve</td>
<td>04</td>
<td>43</td>
</tr>
</tbody>
</table>

Table 04: Sensitivity, Specificity, PPV, and NPV of three Analytical Staining Methods for H. pylori Infection in 95 Patients

<table>
<thead>
<tr>
<th>Diagnostic Methods</th>
<th>Sensitivity in %</th>
<th>Specificity in %</th>
<th>Positive predictive value (PPV)</th>
<th>Negative predictive value (NPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H &amp; E stain</td>
<td>94.44%</td>
<td>100%</td>
<td>100%</td>
<td>85.19%</td>
</tr>
<tr>
<td>Giemsa stain</td>
<td>94.12%</td>
<td>99.65%</td>
<td>99.46%</td>
<td>84.42%</td>
</tr>
<tr>
<td>IHC</td>
<td>94.74%</td>
<td>100%</td>
<td>100%</td>
<td>94.42%</td>
</tr>
</tbody>
</table>
DISCUSSION

Ever since this spiral germ was discovered in gastritis patients, pathologists of every era have poured in their heart and soul to come up with the best diagnostic techniques. As a result, various invasive and non-invasive tools have been established to identify the H. pylori efficiently and have become a part of the routine practice. Staining has been regarded as one of the most valuable diagnostic tools when it comes to H. pylori associated gastritis, and various types of stains are being used to complement such diagnosis. Our research is also aimed at further streamlining the diagnostic process by checking the performance of different stains in terms of specificity and sensitivity, with their positive & negative predictive values.

Our study has been featured with the specificity and sensitivity values of different stains. Most hospitals rely on the staining of gastric antral biopsies with H&E stain as it is the most reasonable and reliable analytical method for H. pylori. We recorded 100% specificity and 94.4% sensitivity for the H & E stain in our study, while this range falls between 87.90% and 69.93% respectively in various other studies. The use of high magnification H & E stain results in making the H. pylori directly identifiable to evaluate the degree of inflammation. However, seeing the organism becomes more of a challenge when there is a combination of low-density H. pylori and atrophic mucosa.

We recorded the specificity and sensitivity of Giemsa, another popular stain for histological examination of gastric biopsies, to be at 95.65% in our study. Many researchers prefer Giemsa staining because it offers ease of use, affordability and consistency in results.

IHC stain tops the ladder in this study with 100% specificity and 94.74% sensitivity, followed by the H & E and Giemsa stain. IHC has been considered as the most sensitive and reliable stain by many researchers, as also indicated by the results of our study. IHC stain is considered especially advantageous in partially treated patients of H. pylori gastritis, a setting with the possibility of resulting in un-common (including coc cocoid) forms, capable of mimicking bacterial micro-organisms or cellular debris on H & E staining. Less screening time and high specificity features serve as the biggest benefit of IHC stain, helping it exclude other similar-shaped organisms.

Moreover, the interpretation of rare H. pylori in a smear is quite problematic, more so when the background is dirty.

As demonstrated in one of the studied cases, Helicobacter is mostly hosted inside or under the surface mucus layer, an area susceptible to partial loss while processing the sample for histological examination. This probability further increases when there is a low bacterial count. These findings suggest that in case of low bacterial loads, using more than one analytical method is more feasible to suppress the false-negative rate.

CONCLUSION

Histologically, the appearance of H. pylori was more frequent in chronic active gastritis cases as compared to chronic gastritis cases. The three different staining methods resorted to in this study displayed equal efficiency in terms of specificity, sensitivity, PPV and NPV. With 100% PPV, IHC stain came up with the highest sensitivity amongst all stains. Similarly, the value of the H & E stain remained much closer to the IHC stain. Thus, in order to cut on the false-negative rate, more than one diagnostic method should be used for cases having low amounts of H. pylori.

Author’s Contributions: RAL: designing the study and data analysis. OAT: Microscopic examination. SS: Manuscript drafting. SS: help in final draft and approval from all authors. SHS: provision of endoscopic biopsies. NN: manuscript revision and critical analysis

REFERENCES