Comparison Between Immunohistochemical Marker and Conventional Histochemical Stains in Detecting Helicobacter pylori

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Abstract

Introduction: The role of H. pylori in causing gastroduodenal diseases has been extensively investigated, as, it is incriminated as a potential major cause of peptic ulcers (95% of duodenal ulcers, 85% of gastric ulcers), atrophic gastritis (a precursor lesion of cancer), accordingly, International Agency for Cancer Research explicitly declared H. pylori as a carcinogenic element.

Objectives: This study was aimed at evaluating the role of immunohistochemical marker for identifying H. pylori, and further comparing various conventional histochemical staining techniques used for detecting H. pylori, namely, (Giemsa and Warthin and Starry with IHC) in biopsy specimens.

Materials and Methods: Fifty archives formalin- fixed paraffin- processed blocks were prepared, and then 5µm sections were cut and stained by: Giemsa, Warthin and starry stains and immunohistochemistry. The results and observations were organized and interpreted in light of clinical and laboratory findings.

Results: A total of 50 archive blocks that, obtained from 50 patients. Among whom there were 30 (60%) females and 20 (40%) males, with ages ranging between 15 to 95 years. On staining with Immunohistochemistry, 29 cases were found positive. In all cases the bacteria were clearly and easily interpreted. The sensitivity and specificity of Giemsa stain were found to be 58.6%, 100% correspondingly, whereas the silver method shows sensitivity and specificity as 34.5% and 100% respectively. The presented results of the current study were statistically significant compared to the gold standard methods i.e. immunohistochemistry (P-value < 0.005), remarkably, the positive predictive value of Giemsa and silver stains were found to be 100%. Nevertheless, the negative predictive value of Giemsa and silver stains were found to be (63.3%, 52.2%) respectively.

Conclusions: IHC is highly sensitive and acumen, but it is quite difficult in routine and daily basis work. It could be applied in special cases for instance (wherein negative results with other histochemical stains, after treatment whereupon bacteria decreased in number or metamorphosed into coccoid form. Interestingly, the use of Giemsa is strongly recommended as it’s much faster, cheaper, easier in interpretation and more reproducible.

Keywords: H. pylori; Immunohistochemical Stain; Giemsa Stain; Warthin and Starry Stain
Introduction

*H. pylori* is spiral, microaerophilic, gram negative rod [1]. It is isolation is traced back to 1982 thanks to Marshall and Warren [2], following that exhaustive researches have been done to study the mainstay role played by these bacteria in inducing gastroduodenal diseases [3-7]. Furthermore, it considered as major cause of peptic ulcers (95% of duodenal ulcers, 85% of gastric ulcers, atrophic gastritis (a precursor lesion of cancer) [8] and culminated in 1994 whereby the International Agency for Cancer Research has declared that *H. pylori* as a potential carcinogenic element [9].

Prominently, Bacteria exhibit narrow range of organ [10], where of can persist lifelong unless treated by suitable antibiotic indicating that bacteria possess tactical mechanisms enabling it to adapt and withstand the environment of stomach. Unsurprisingly, the paramount important one is the enzyme urease which degrades the urea and neutralizes the acidity of stomach. Not to mention, the urease constitutes 10% of all bacterial proteins) [11]. Notably, the main important route of transmissions are, oral-oral route, oral-fecal route, and rarely iatrogenic route (via endoscopy or other surgical materials) [12,13]. Surprisingly, the exact mechanisms of pathogenesis are not yet clear. However, numerous studies have described the sequential of effects as, ensuing bacterial colonization the immune response is triggered producing and recruiting inflammatory components, principally, and predominantly Th1, which consequently, induce acute gastritis which dramatically, evolve into chronic active gastritis [14,15]. Importantly, these conditions are mostly asymptomatic and prolonged leading to excessive cellular damage and exponential proliferation that result in DNA damage and developing cancer [16]. Notwithstanding, groundbreaking studies have revealed the lack of a crystal-clear relationship pertaining bacteria with the unit infections indicating that, there are other factors that, might play significant role in pathological conditions associated with bacteria [17-19]. Hence there is a reasonable concordance between these factors and geographic distribution.

In Asian countries by far the impact of bacteria varies greatly, e.g. these bacteria pose a pivotal role in gastritis and cancers [20-23], whereas in Africa although it is highly prevalent of *H. pylori* there are low prevalence of gastric cancer [24,25], this may be ascribed to differences among genetic strains distributed in various countries, additionally, other factors contribute in pathogenesis are, host susceptibility, immunological response and diet [26]. Unfortunately, treatment of *H. pylori* is an emerging problem, owing to an increasing in antibiotic resistance [27,28]. Hopefully, there are many methods used for detecting *H. pylori* in the stomach some of them depends upon the endoscopy (invasive tests) by obtaining biopsy that, subsequently stained using chemical stains, molecular technique using polymerase chain reaction (PCR), rapid urease test (RUT), and immunohistochemical tests, other not depend on the endoscopy (not invasive) such as serological tests (serological tests in the blood and stool) or genetic tests like PCR in stool [29].

Materials and Methods

This was a descriptive, preliminary, retrospective, cross sectional, and hospital-based study, aimed to study *Helicobacter pylori*, thereby 50 formalin-fixed, paraffin-embedded endoscopic archive blocks obtained from patients with upper gastrointestinal symptoms, in a period between November 2014 till June 2015 were retrieved from archives of departments of histopathology in Military Hospital, Omdurman Teaching Hospital, Khartoum teaching Hospital, and Al-Ribat National Hospital. 5-μm histological sections were prepared and stained with Giemsa, Warthin and Starry and an anti-*H. pylori* antibody immunostaining. The stained sections were double blind evaluated by pathologists independently.

Warthin and starry stain [30]

1% silver nitrate was applied in deparaffinized sections for 30 seconds in Microwave. Then the developer solution (5% Gelatin + 0.3g of Hydroquinone in 10 ml of sodium acetate solution at pH 3.6 + 2% silver nitrate) for 90 seconds in Microwave. then washed three times in D.W at room temperature, dehydrated, Cleared and mounted in DPX.
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**Giemsa Stain** [31]

Deparaffinized sections were stained with the working solution of Giemsa (methanol, Giemsa solution and Distilled water). Then Differentiated in 1% acetic acid, then washed in water and left to dry at room temperature and mounted by DPX. The bacteria stained with magenta color while the background stained blue.

**Immunohistochemistry Technique** [32]

It was performed on 5-μm sections of all formalin-fixed, paraffin–embedded biopsy specimens. Known *H. pylori* contained section was used as positive control while primary antibody was omitted from another section thus used as negative control.

**Pretreatment of Section (Antigen Retrieval)** [32]

Antigens were retrieved using (US Pat Nos5, 244, along with their foreign equivalents), in citrate buffer solution at 97°C for 10 minutes. Then blocked by 3% Hydrogen peroxide in methanol (HK111-5KT) at humidified chamber for 20 minutes then blocked using Bovine serum Albumin (power block HK 083-5K). A rabbit polyclonal antibody (from tissue culture supernatant diluted in PBS, pH 7.6 containing 5% BSA and 0.09% Sodium azaide) against *H. pylori* was applied for 40 mins, then washed in Buffer solutions for 5 minutes, then polymer solution was applied for 15 minutes, then washed in buffer for 5 mins, chromogen solution was added for 10 minutes, subsequently washed in D.W.

**Assessment of stains** [34]

Using role of *Thumb*, the stained slides were ascertained and assessed by blind double pathologists, and slides were rated according to the quality of stain as follow:

- 8 – 10......... excellent staining quality
- 5 – 7 ............. good staining quality
- Less than 5............ bad staining quality

Then the obtained data were statistically analyzed using one-way ANOVA test.

Statistical Package for Social Science (SPSS-v17) was used to analyze the applied data and to perform Pearson Chi-square test for statistical significance (P-value).

**Results**

The study was designed to identify *H. pylori* among 50 patients. Among whom there were 30 (60%) females beside 20 (40%) males. Their ages ranged between 15 to 95 years with mean age of 50.4 year. On basis of pathological diagnosis, they were categorized as follow, 34% were originally diagnosed as adenocarcinoma, 42% as chronic gastritis, 12% chronic inflammation, 4% acute gastritis, 3% atrophy, while 1% has been diagnosed as peptic ulcer. Regarding the distribution of our study population by age, sizable people were found within age group 31 to 60 (58%). Concerning the association of age group and pathological significance our findings were statistically show no major difference. Additionally, when comparing the distribution of gender by pathological conditions the results were found to be insignificant as well.

On comparing conventional histochemical techniques, *H. pylori* was fairly and squarely identified in 17 (58.6%) Giemsa stained sections. Furthermore, *H. pylori* could be easily and well identified in Warthin-Starry stained sections; 10(34.5%) were strongly positive. Nonetheless on staining with Immunohistochemistry, 29 cases were found to be positive. Remarkably in all cases the bacteria were conspicuous, prominent and easily detected in the immune-stained sections compared to conventional histochemical methods. On comparing immunohistochemical to conventional Histochemical methods.

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The sensitivity and specificity of Giemsa stain were found to be 58.6%, and 100% respectively. In discordance to the silver method which shows sensitivity about 34.5% and 100% specificity, the results were shown to be significant when compared to the gold standard methods i.e. immunohistochemistry (P < 0.05). Whereas positive predictive value of Giemsa and silver stains were calculated as 100%, nonetheless, the negative predictive value of Giemsa and silver stains have been calculated as (63.3%, 52.2%) respectively.

**Figure 1:** *H. pylori in gastric mucosa using Giemsa staining method (X 100).*

**Figure 2:** *H. pylori using Warthin_Starry staining method (X 100).*
Discussion

Early and accurate diagnosis of H. pylori offers not only better treatment, but also the wellbeing of the patients. Among all tests and methods of diagnosis, the most preferred one is the histological examination (examination of biopsy from the stomach) [33]. Although it is slow, laborious, more expensive, show some drawbacks in certain cases like atrophy which may lead to false negative results [34], and it associated with high observation variability [35], it gives useful information about the conditions associated with bacteria. Furthermore, histological examination of biopsies gives plausible information about the development of disease status, response to treatment, and provide a good opportunity for detecting inactive phase of bacteria (coccoid form) [36,37]. Accordingly, various histological techniques have been used. In the present study conventional histochemical stains namely, (Giemsa and Warthin and Starry) show low sensitivity, which might be imputed to the number of bacteria, accordingly, there is a real needful for large number of bacteria to display positive results, unfortunately, these stains have failed to demonstrate the coccoid form of bacteria which has meticulously, and crisply been demonstrated via IHC, this finding also shown by evolving body of studies [38,39].

Furthermore, in study conducted Loffeld, et al. [40]. has compared between IHC, Giemsa and culture in terms of sensitivity and they reported that culture is the least sensitive one and IHC is the more sensitive one, Unhappily, we got difficulties in interpreting the results particularly, with Warthin and Starry's stain although the bacteria appear larger than in Giemsa and IHC as well, there are several components in tissue may have badly picked silver stain like (argyrophilic infected cells by cytomegalovirus, gastrointestinal tract argentaffin beside argyrophill cells) [41]. Not surprisingly, the aforementioned components affect the contrast and wherefore making the interpretation of results more problematic. Additionally, silver needs much time for preparation as much as staining procedures. Needless to mention that, staining solution can quickly get contaminated. Moreover, the solution is highly precipitated in slides; thereby highly precaution

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measures are strongly needed. Nonetheless, Giemsa is easy in the preparation as much as the staining protocol is much easier and faster. Interestingly, conventional histochemical stains confer high specificity as noticed in both Giemsa and Warthin and Starry. Consistently, Laine, *et al.* [42] and Fallone, *et al.* [43] have reported in their studies high specificity of histochemical stains. Additionally, Ashton, *et al.* [39], unequivocally found the latter techniques generate false positive results. This may be ascribed to the differences in the preparation of stains and staining procedures employed from one place to another, in addition to interobserver variability in the ascertainment and assessment of results and variation from batch to batch in histochemical stains could lead to these variations as reported by many [35]. However, many of the researches done to determine the specificity and sensitivity of Giemsa stain give good results [44,45], also it highly reproducible and cost effective, anyhow IHC reported by many as sensitive, specific and easy in interpreting of *H. pylori* specially when using heat induced retrieval because it decreased the background stain, therefore give good contrast [35,38,39,46,47]. Nonetheless, IHC higher in cost and not widely available specially in developing countries.

**Conclusion**

In conclusion IHC highly sensitive and specific, but difficult to be applied in every biopsy (take more time, cost and training ), but we can apply it in special cases like (negative results with other histochemical stains, after treatment where bacteria decrease in number or appear in coccoid form and in cases of lymphoma where the treatment of *H. pylori* is critical in regression of lymphoma), however the use of Giemsa is recommended because it more fast, cheap, easy in interpretation and therefore it more reproducible.

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