ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

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

Eman Taha Ali[1]*, Tagwa Mohamed Fadul[1], Marwa Ali Nasr[1], Emmanuel Edwar Siddig[2,3,4], Nouh S. Mohamed[5,6] and Ali Mahmoud Mohammed Edris[1,7]

[1]*Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan*

[2]*Faculty of Medical Laboratory Sciences, Unit of Basic Medical Sciences, University of Khartoum, Sudan*

[3]*Mycetoma Research Center, University of Khartoum, Sudan*

[4]*Nile College, School of Medicine, Sudan*

[5]*Departments of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Nile College, Khartoum, Sudan*

[6]*National University, National University Research Institute, Khartoum, Sudan*

[7]*Faculty of Applied Medical Sciences, University of Bisha, Bisha, Kingdom of Saudi Arabia*

***Corresponding Author**: Eman Taha Ali, Department of Histopathology and Cytology, Faculty of Medical Laboratory Sciences, University of Khartoum, Sudan.

Received: May 28, 2018; **Published:** July 23, 2018

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 to1982 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 n(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.

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 (U.S 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.

(Giemsa and Warthin Starry) the results were calculated using ANOVA test, the results have shown to be significant using IHC as a gold standard method ($P < 0.05$).

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.

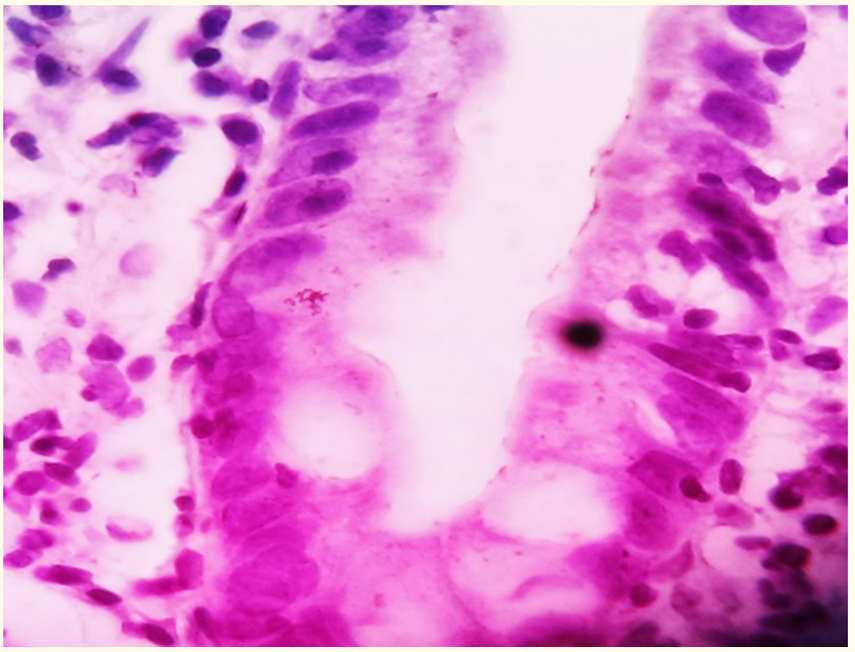

Figure 1: *H pylori in gastric mucosa using Giemsa staining method (X 100).*

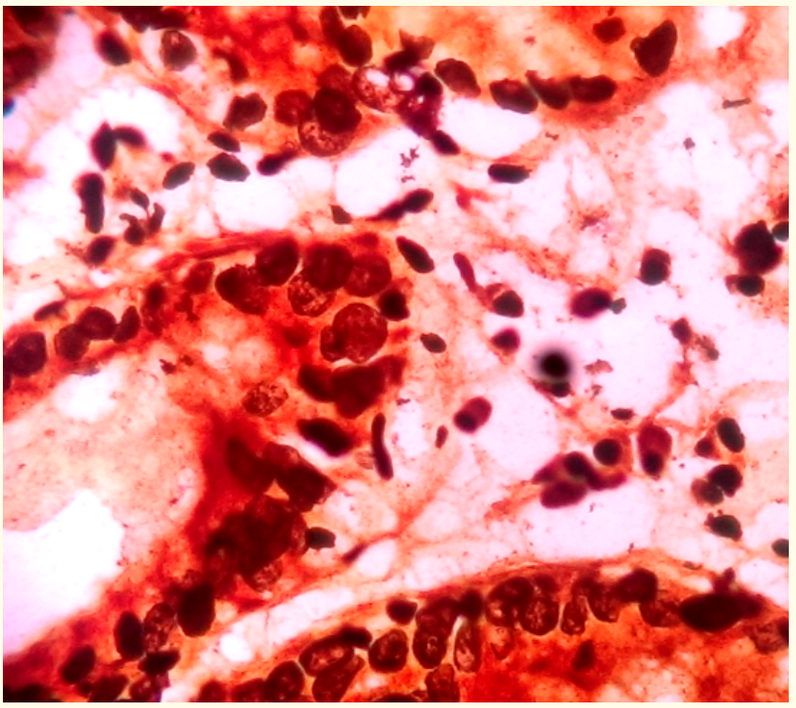

Figure 2: *H. pylori using Warthin_Starry staining method (X 100).*

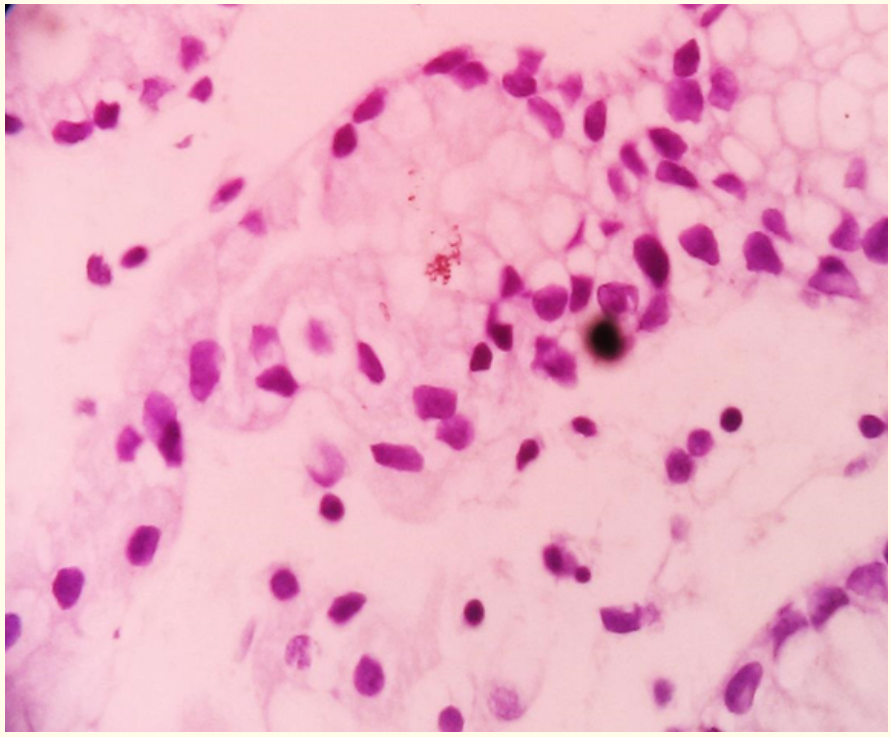

Figure 3: *H. pylori using IHC (X 100).*

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

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.

Bibliography

1. Furchtgott L., *et al*. "Mechanisms for maintaining cell shape in rod-shaped Gram-negative bacteria". *Molecular Microbiology* 81.2 (2011): 340-353.

2. Marshall BJ and Warren JR. "Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration". *Lancet* 1.8390 (1984): 1311-1315.

3. LI X., *et al*. "Role of bile reflux and Helicobacter pylori infection on inflammation of gastric remnant after distal gastrectomy". *Journal of Digestive Diseases* 9.4 (2008): 208-212.

4. Pajares J and Gisbert J. "Helicobacter pylori: its discovery and relevance for medicine". *Revista Española de Enfermedades Digestivas* 98.10 (2006).

5. Vaira D., *et al*. "*Helicobacter pylori*: Its Role in Gastric Disease". *Digestive Diseases* 8.6 (1990): 322-336.

6. Dixon M. "Commentary: Role of Helicobacter pylori on Gastric Mucosal Damage, Gastric Cancer, and Gastric MALT Lymphoma". *Gastroenterology* 113.6 (1997): S65-S66.

7. LI X., *et al*. "Role of bile reflux and Helicobacter pylori infection on inflammation of gastric remnant after distal gastrectomy". *Journal of Digestive Diseases* 9.4 (2008): 208-212.

8. Koch M., *et al*. "47 PS Functional dyspepsia and helicobacter pylori (HP): Is eradication clinically relevant? A meta-analysis on the natural history of gastritis and gastric and duodenal ulcers". *Digestive and Liver Disease* 34.1 (2002): A22.

9. Anonymous Live flukes and Helicobacter pylori. "IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon, 7-14 June 1994". IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 61 (1994): 1-241.

10. Waluga M. "From the stomach to other organs: Helicobacter pylori and the liver". *World Journal of Hepatology* 7.18 (2015): 2136.

11. Davis GMobley H. "Contribution of dppA to Urease Activity in Helicobacter pylori 26695". *Helicobacter* 10.5 (2005): 416-423.

12. Brown L. "Helicobacter Pylori: Epidemiology and Routes of Transmission". *Epidemiologic Reviews* 22.2 (2000): 283-297.

13. Buzás G. "Gastric tubes as vectors of Helicobacter pylori transmission". *Medical Hypotheses* 75.1 (2010): 47-49.

14. Hunt R and Tytgat G. "Helicobacter pylori". Dordrecht: Springer Netherlands (1996).

15. Kim WMoss S. "The role of Helicobacter pylori in the pathogenesis of gastric malignancies". *Oncology Reviews* 2.3 (2011): 131.

16. Mishra S., *et al*. "Prevalence of Helicobacter pylori in asymptomatic subjects-A nested PCR based study". *Infection, Genetics and Evolution* 8.6 (2008): 815-819.

17. Olivares D and Gisbert J. "Factors involved in the pathogenesis of Helicobacter pylori infection". *Revista Española de Enfermedades Digestivas* 98.5 (2006).

18. Backert SClyne M. "Pathogenesis of Helicobacter pylori Infection". *Helicobacter* 16 (2011): 19-25.

19. Honarmand-Jahromy S. "Reciprocal impact of host factors and Helicobacter pylori genotypes on gastric diseases". *World Journal of Gastroenterology* 21.31 (2015): 9317.

20. Singh K. "Causal role of Helicobacter pylori infection in gastric cancer: An Asian enigma". *World Journal of Gastroenterology* 12.9 (2006): 1346-1351.

21. Wang R. "Helicobacter pylori infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China". *World Journal of Gastroenterology* 8.6 (2002): 1103.

22. Wong B., *et al*. "Differential Helicobacter pylori infection rates in two contrasting gastric cancer risk regions of South China". *Journal of Gastroenterology and Hepatology* 14.2 (2002): 120-125.

23. Matsuo T., *et al*. "Low Prevalence of Helicobacter pylori-negative Gastric Cancer among Japanese". *Helicobacter* 16.6 (2011): 415-419.

24. SEGAL I., *et al*. "Helicobacter pylori: the African enigma". *Gut* 43.2 (1998) :300-301.

25. Tsuji S. "The "Costa Rican enigma" of Helicobacter pylori CagA and gastric cancer". *Journal of Gastroenterology* 41.7 (2006): 716-717.

26. Fuccio L. "Gastric cancer, helicobacter pylori infection and other risk factors". *World Journal of Gastrointestinal Oncology* 2.9 (2010): 342-347.

27. Helicobacter pylori: Optimizing treatment of Helicobacter pylori infection". *Nature Reviews Gastroenterology and Hepatology* 12.3 (2015): 123-123.

28. O'Connor A., *et al*. "Treatment of Helicobacter pylori Infection 2011". *Helicobacter* 16 (2011): 53-58.

29. McNulty C., *et al*. "Diagnosis of Helicobacter pylori Infection". *Helicobacter* 16 (2011): 10-18.

30. Churukian CSchenk E. "A Warthin-Starry Method for Spirochetes and Bacteria Using a Microwave Oven". *Journal of Histotechnology* 11.3 (1988): 149-151.

31. Gray S., *et al*. "Simplified techniques for identifying Campylobacter pyloridis". *Journal Clinical Pathology* 39.11 (1986): 1279.

32. Immunohistochemistry Consumables and Reagents (2016).

33. Chey Wong B. "American College of Gastroenterology Guideline on the Management of Helicobacter pylori Infection". *The American Journal of Gastroenterology* 102.8 (2007): 1808-1825.

34. Lee J., *et al*. "Optimal Biopsy Site for Helicobacter pylori Detection during Endoscopic Mucosectomy in Patients with Extensive Gastric Atrophy". *Helicobacter* 17.6 (2012): 405-410.

35. ANDREW A., *et al*. "Observer variation in the assessment of chronic gastritis according to the Sydney system". *Histopathology* 25.4 (1994): 317-322.

36. LIN S., *et al*. "A comparison of diagnostic tests to determine Helicobacter pylori infection". *Journal of Gastroenterology and Hepatology* 7.2 (1992): 203-209.

37. She F. "Virulence and potential pathogenicity of coccoid Helicobacter pylori induced by antibiotics". *World Journal of Gastroenterology* 7.2 (2001): 254.

38. Jonkerst D., *et al*. "Evaluation of immunohistochemistry for the detection of Helicobacter pylori in gastric mucosal biopsies". *Journal of Infection* 35.2 (1997): 149-154.

39. Ashton-Key M., *et al*. "Detection of Helicobacter pylori in gastric biopsy and resection specimens". *Journal of Clinical Pathology* 49.2 (1996): 107-111.

40. Loffeld R., *et al*. "Helicobacter pylori in gastric biopsy specimens. Comparison of culture, modified Giemsa stain, and immunohistochemistry. A retrospective study". *The Journal of Pathology* 165.1 (1991): 69-73.

41. Tamate H and Kondo Y. "An Improved Staining of the Three Fundic Gland Cells of Mammalian Stomach". *Stain Technology* 36.4 (1961): 254-255.

42. Laine L., *et al*. "Prospective comparison of H and E, Giemsa, and Genta stains for the diagnosis of Helicobacter pylori". *Gastrointestinal Endoscopy* 45.6 (1997): 463-467.

43. Fallone C., *et al*. "Haematoxylin and Eosin Staining of Gastric Tissue for the Detection of Helicobacter pylori". *Helicobacter* 2.1 (1997): 32-35.

44. Choi Y., *et al*. "Accuracy of Diagnostic Tests for Helicobacter pylori in Patients with Peptic Ulcer Bleeding". *Helicobacter* 17.2 (2012): 77-85.

45. Rotimi O. "Histological identification of Helicobacter pylori: comparison of staining methods". *Journal of Clinical Pathology* 53.10 (2000): 756-759.

46. Wang X., *et al*. "The role of routine immunohistochemistry for Helicobacter pylori in gastric biopsy". *Annals of Diagnostic Pathology* 14.4 (2010): 256-259.

47. Tokunaga Y., *et al*. "Modified rapid urease test for Helicobacter pylori detection in relation to an immunohistochemical stain". *Journal of Gastroenterology and Hepatology* 15.6 (2000): 617-621.

Volume 14 Issue 8 August 2018
©All rights reserved by Eman Taha Ali., *et al*.