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Role of Immunohistochemistry in Detection of Helicobacter Pylori in Conjunction with Special Stains in Gastric Biopsy

Dr. M.G. Bhuvaneswari[1] & Dr. Dhanalakshmi Arumugam[2]
[1]*Assistant Professor of Pathology, Coimbatore medical college and hospital, Coimbatore, Tamilnadu, India*
[21]*Associate Professor of Pathology, Coimbatore medical college and hospital, Coimbatore, Tamilnadu, India*

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ABSTRACT
BACKGROUND AND OBJECTIVES: H.pylori infection is the most common cause of gastritis worldwide and most prevalent in developing countries like India.Hence the need of low cost, easy procedure and easily available test to identify the bacilli in initial stages of infection and early treatment. Several special stains and immunohistochemistry is available. This study compared the Toludine blue and Giemsa stain with the gold standard IHC method. Aim of our study is to identify the Helicobacter pylori organism in gastric biopsy by using two special stains giemsa and toludine blue, to study the role of immunohistochemistry in detection of H.Pylori and to compare the efficacy of the two special stains with immunohistochemistry.**MATERIALS AND METHODS:**This study was conducted on 75 cases for a period of one year. H.pylori was identified by using two special stains, Giemsa and Toludine blue. Immunohistochemistry was done to confirm the H.pylori positivity in gastric biopsy. **RESULTS:**Out of 75 cases with mean age of 43years, H.pylori positivity were identified by IHC in 53 cases (71%), by Giemsa in 35 cases(47%) and by Toludine blue in 42 cases(56%). Both stains showed significant p value <0.05. As compare with Giemsa stain, Toludine blue is cheaper and easy to perform. Most of the mild bacterial colonization (40%) were identified by IHC.**CONCLUSION:** We concluded both Giemasa and Toludine blue has been proved to be a reliable one in identification of H.pylori. But Toludine blue is more economical and easy to perform. Immunohistochemistry is the most reliable one in identification of mild colonization, modified coccoid form and bacilli obscured by mucus and debris.

KEY WORDS: Helicobacter pylori, Giemsa stain, Toludine blue stain, Sydney system and Immunohis to chemistry.

I. INTRODUCTION

The bacteria found by Giemsa stain were still responsible for the greatest number of patients with a positive diagnosis of infection, regardless of the existence of various degrees of gastritis activity[1]. The imperative of eradicating H. Pylori is increasingly accepted by doctors as part of the treatment of peptic ulcer disease and gastric cancer prevention. In the meantime, a new and successful therapeutic regimen for H.pylori is becoming available, proof of eradication is important and the histopathological analysis of post-treatment biopsy specimens is best collected[2]. Helicobacter pylori organism is the most common cause of chronic gastritis. H.pylori colonization of the gastric mucosa is related with gastritis, gastric peptic ulcer, gastric adenocarcinoma and gastric lymphoma.[3,4,5]. Infected person have three to six fold greater risk of developing gastric cancer. Recently WHO classifies this as a CLASS I carcinogen. H.pylori associated gastric inflammation leads to development of gastric carcinoma such as gastric atrophy, intestinal metaplasia, dysplasia and carcinoma lastly. So early detection is becomes essential to completely eradicate this organisms and thereby prevents the dreadful consequence of carcinoma by using triple therapy.

H.pylori seen in the gastric pits and overlying mucus. H.pylori colonization of inflammatory response with an initial development of acute gastritis, which when prolonged leads to chronic inflammation. Chronic inflammation induced by bacteria causes the loss of normal architecture of gastric mucosa H.pylori is present only in the apical portion of gastric foveolar glands and not in the gland cytoplasm is termed superficial chronic active gastritis. Usually H.pylori colonisation triggers an inflammatory reaction in the lamina propria. Mucosal neutrophils are a distinctive histological feature of H.pylori infection. The grading of activity in gastritis is done according to the MODIFIED SYDNEY SYSTEM. [6]

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H.pylori is a spiral-shaped, flagellated, Gram-negative rod, microaerophilic bacterium [7] is one of the commonest bacterial infections throughout the world. It involves 50% of population in industrialized countries [8,9,10]. In developing countries like India it involves up to 80-90% of the population [11].

Different modalities were used in the diagnosis of H. pylori and they grouped into two categories:

1) Invasive tests, which need endoscopy such as culture, rapid urease test, histology and PCR.
2) Non-invasive tests, which does not require endoscopy, such as serology, urea breath test, and fecal antigen test [12, 13].

Commonest and most sensitive of all methods is histological detection. It has the advantage to evaluate pathological changes associated with H. pylori infection, including inflammation, atrophy, intestinal metaplasia and carcinoma [14]. There are many histochemical stains used for histological detection of H. pylori in gastric biopsies and resections, including hematoxylin and eosin (H&E), giemsa, warthin starry silver stain, Modified Giemsa, genta, and toluidine blue. H&E and Giemsa are routinely staining methods, which are used in pathological laboratories for detection of H. pylori. Toluidine blue stain is simple, cheap and easy to perform.None of these are more specific for the organism and the coccoid forms and indolent forms are missed out easily in routine stains. Recently IMMUNO HISTOCHEMISTRY, IN SITU HYBRIDISATION & PCR has been proposed as alternative & more specific modalities in detection.

The present study was planned i) to compare H&E, Giemsa and toluidine blue staining in the detection of H.pylori along with immunohistochemistry detection. (ii) To compare the results of these staining methods in different groups of patients due to the degree of inflammation and activity.

II. MATERIALS AND METHODS

This study was done in the Department of Pathology along in conjunction with Medical Gastroenterology Department, at Coimbatore medical college and hospital, Coimbatore for a period of one year with a sample size of 75 cases. Co-operative patients from age 18 to 70 years were included while the uncooperative patients with age less than 18 and more than 70 years were also excluded. Tiny and inadequate specimen and autolyzed specimens were also excluded.

Data Collection

Total of 75 cases were studied. Endoscopy was done by using Olympus GIFSQ 30 video endoscopy system in Department of Medical Gastroentrology at Coimbatore medical college and hospital. Endoscopy findings and patient clinical presentation were recorded in a standard proforma . Biopsy was taken from antrum and body of the stomach and immediately placed in 10% formalin for fixation.

Methodology

Samples were received in our department with records and registered. The specimens were processed routinely. Four microns sections were taken from both the tissues and stained with hematoxylin and eosin. The histolopathological changes were analyzed such as inflammation, glandular atrophy, neutrophilic activity, intestinal metaplasia and H.pylori status. These histolgic changes was graded according to the Sydney system.

Special stains giemsa and toludine blue were done subsequently to detect the organism and intensity were graded as per the Sydney system. Immunohistochemistry was done using rabbit polyclonal anti-H.Pylori antibody from Biogenex. The organism was easily identified by brown colour against blue background and the intensity was graded. IHC positive cases were considered as H.pylori positive. All the findings were recorded. The efficacy of the two stains were compared in conjunction with IHC for the detection of H.pylori organisms.

III. IMMUNOHISTOCHEMISTRY

Section Cutting

The sections were cut at 4microns thickness from paraffin embedded blocks, floated on to Poly – L – Lysine coated slides and incubated at 37ºc for one day and further incubated at 58ºc for overnight. It is very important not to allow sections to dry at any stage of the procedure and to carry out the steps of incubation with antibody at 37ºc.

Antigen Retrieval

Many methods have been used for antigen retrieval including pressure method, microwave retrieval, water bath, autoclave and proteolytic enzyme digestion. In our institution we used microwave antigen retrieval method. (Heat mediated antigen retrieval technique).

Buffer

Citrate buffer (0.01M) at pH of 6.

Immunohistochemistry Technique

Two step indirect method.

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IV. RESULTS AND DISCUSSION

This present study was done in75 patients who attended the Medical Gastroenterology Department of Coimbatore Medical College with complaints of nausea, vomiting, dyspepsia and abdominal pain. Majority of the patients 63% were presented with features of gastritis.

In the total 75 cases, 53 showed H.Pylori positive. Among the 53cases males were 34 (45%) and females were 19 cases (25%). The graphical representation of this is shown in Fig. 1. This showed male preponderance of H.Pylori infection.

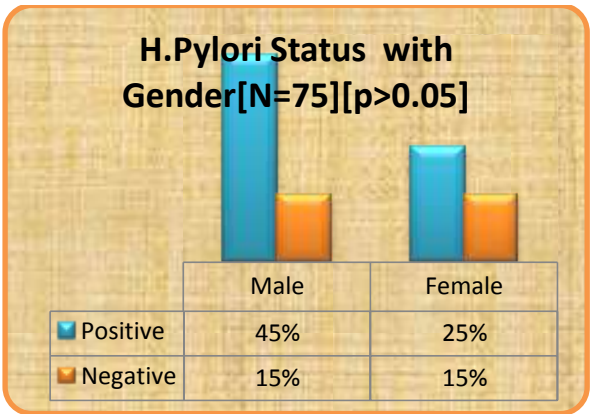

Fig 1. H. Pylori status with gender

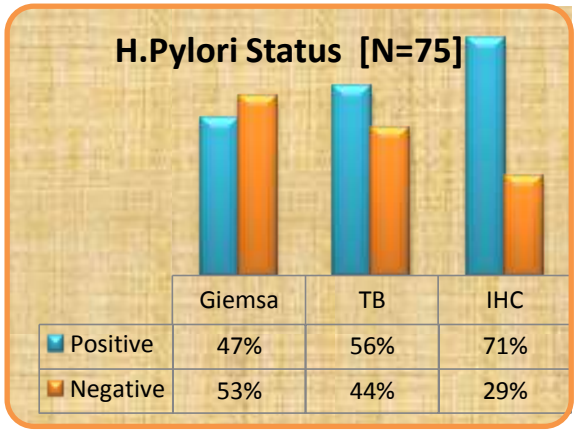

Fig 2. H.Pylori status in Giemsa,TB & IHC

H.Pylori positive status in giemsa, toludine blue and IHC

Fig 2 shows the H.Pylori positive status in Giemsa, TB and IHC This figure shows overall H.pylori positivity among total 75 cases. Immunohistochemistry showed positive in 53 cases (71%). Giemsa stain showed positive in 35cases(47%) and toludine blue in 42cases (52%) whose numerical values are given in Table 1.

Table 1 H.Pylori positive status in Giemsa, TB and IHC

H.pylori status	Giemsa	TB	IHC
Positive	35	42	53
Negative	40	33	22
Total	75	75	75

Comparison of Giemsa Stain with IHC

As comparing with IHC, Giemsa showed true positive in 31 cases out of total positive in 53 cases. False positive was noted in 22 cases, false negative in 4 cases. Statistical analysis was done. It revealed sensitivity of 88.6%, specificity of 45% with a positive predictive value of 58.5% and negative predictive value of 81.8%. The p value was <0.01 and that was significant.

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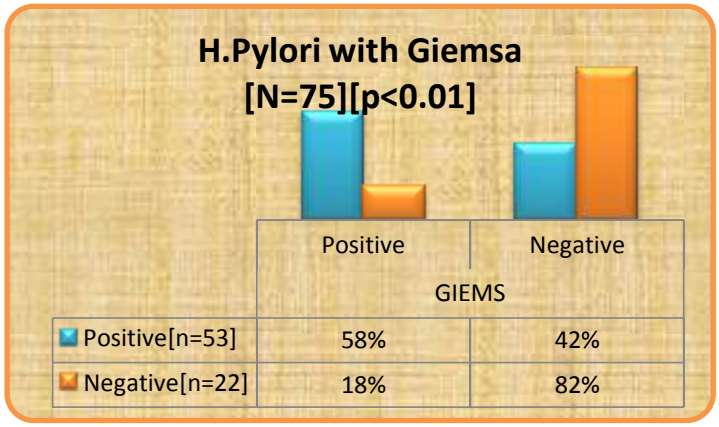
	GIEMSA		
H.Pylori Status	**Positive**	**Negative**	**Total**
Positive	31	22	53
Negative	4	18	22
Total	35	40	75
Sensitivity - 88.6%			
Specificity - 45.0%			
PPV - 58.5%			
NPV-81.8%			

Table 2. Giemsa stain with IHC

Fig 3 Comparison of Giemsa stain with IHC



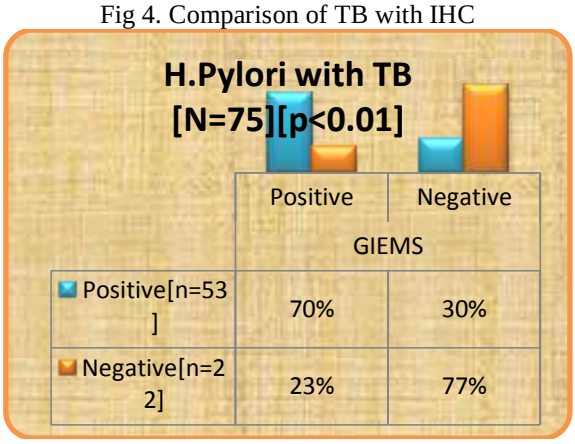
Comparison of Toludine Blue stain with IHC

Out of 53 positive cases which was confirmed by IHC, Toludine blue showed true positive in 37 cases, false positive in 16 cases and false negative in 5 caises. It revealed sensitivity of 88.1%, specificity of 51.5% with a positive predictive value of 69.8% and a negative predictive value of 77.3%. Its showed significant p value of <0.01%. The comparison of TB stain with IHC values are represented in Table 3 and Fig 4.

Table 3 TB stain with IHC

	TB		
H.Pylori Status	**Positive**	**Negative**	**Total**
Positive	37	16	53
Negative	5	17	22
Total	42	33	75
Sensitivity - 88.1%			
Specificity –51.5%			
PPV –69.8%			
NPV-77.3%			

Fig 4. Comparison of TB with IHC

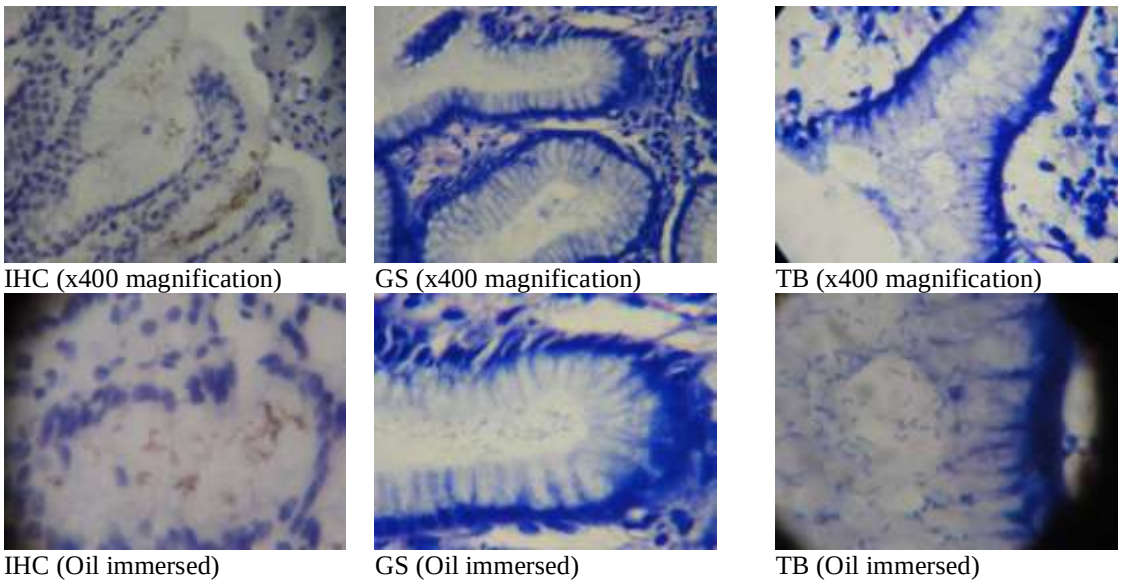


Among the total 75 cases, males are 45(60%) and females are 30(40%) in number. The mean age group was 43years. Minimum age is 20yrs and the maximum age is 70yrs.In the 75 cases, H.Pylori was identified in 53 persons by IHC (gold standard method). The percentage was 76%. Males eare more commonly affected by H.Pylori infections of about 45%, female showed 25% positivity. This study shows male predominance and the maximum number of cases noted in the age group of 41-60 years (48%) and 28% in 20-40 years.

In this present study, two special stains Giemsa and Toludine blue were used for identification of H.Pylori. Confirmatory was done by IHC. Two stains were compared with IHC.

Giemsa stain showed 31 positive cases and 22 negative cases. H.Pylori positivity was 58% positivity in which compare with IHC that showed 76% positivity. It revealed sensitivity of 84.7%, specificity of 45% with appositive predictive value of 58.5% and negative predictive value of 81.8%. 22 cases showed false positive, which is negative in IHC. Statistical analysis of P value was <0.01, which is statistically significant.

Toludine blue stain revealed 42 H.Pylori positive cases and 33 negative cases among 75 cases. H.pylori positivity was 70%. This stain showed sensitivity of 88.1%, specificity of 45% with a positive predictive value of 58.5% and negative predictive value of 81.8% as compared to IHC.Among the 53 cases positive with IHC, toludine blue revealed true positive in 31 cases and false positive in 16 cases. This present study showed more sensitivity in H.pylori identification by using toludine blue than the referral study. H.Pylori images in IHC, GS and TB with magnification are represented in Figure 5.



IHC (x400 magnification) GS (x400 magnification) TB (x400 magnification)

IHC (Oil immersed) GS (Oil immersed) TB (Oil immersed)

Figure 5. H.Pylori images in IHC, GS & TB with magnification and oil immersion

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Comparison between Giemsa and Toludine Blue

Statistical analysis between the two stains in compare with IHC showed both were statistically significant. But Toludine blue showed less false positive rate as compare with Giemsa stain in detection of H.pylori.Toludine blue stain is a simple stain. It is cheap and straight forward procedure. Staining time was 20-30minutes. It is a reliable one in detecting H.pylori as compare with Giemsa stain. The only disadvantage is variable background staining and little contrast between the organisms and tissues.According to our study, we found that Toludine blue has good sensitivity which is close to the sensitivity of Giemsa stain in comparison with IHC.

Role of Immunohistochemistry in H.Pylori Detection

This present study showed 53 positive (76%) H.pylori cases among 75 cases by using polyclonal rabbit anti-H.pylori antibody. H.pylori intensity was graded according to the revised Sydney system as mild, moderate and severe. IHC identified 40% of mild bacterial colonization, but Giemsa and Toludine blue showed 27% and 37% respectively. As compare with the two stains, IHC showed higher reliability in detecting mild form of bacterial colonization and even single bacilli can be detected.

Chronic Inflammation

All cases exhibit variable degree of inflammation in H.pylori infection. As per Sydney system mild inflammation were noted in 39 cases (52%) followed by moderate inflammation in 31 cases (41%) and severe inflammation in 5 cases (7%). H.pylori colonization was seen in 38% of mild inflammation, and 77% moderate inflammation. Our study showed strong relationship between H.pylori colonization and inflammation.

According to **Intisar Pity** Bacterial colonozation was seen in 40% cases of mild inflammation, 72% cases of moderate inflammation and 36% cases of severe inflammation.

Neutrophil Activity

Neutraphil activity was noted in 47 cases out of 75 cases. Modrerate bacterial colonization showed 62% mild neutrophil activity, 23% moderate neutrophil activity. Statistical analysis in compare with bacterial colonization was significant (P value <0.05).

Intestinal Metaplasia

Intestinal metaplasia is commonly associated with severe bacterial colonization. Bacterial colonization seen adjacent to intestinal metaplasia area not in the metapasia area, that suggest alkaline medium prevent the growth of organism.

Glandular Atrophy

In our study, Glandular atrophy mostly seen in severe colonization. 60% of mild glandular atrophy noted in severe bacterial colonization.P value in compare with bacterial colonization was >0.05, that is no significant in this study.So according to our study, Inflammation, neutraphil activity was strongly correlate with bacterial colonization. Intestinal mateplasia was shown to correlate well with severe bacterial colonization, where as mild and moderate intestinal mateplasia was not seen.

V. SUMMARY

H. pylori infection is the most common cause of chronic gastritis. It is a major medical burden worldwide. This is commonly associated with gastric ulcer, peptic ulcer, gastric adenocarcinoma and lymphoma. WHO classifies this organism as class I carcinogen, its infection increase the risk of gastric carcinoma.

Hence early, and accurate detection of this organisms is essential for complete eradication by using triple therapy. Thereby prevents the dreadful consequences. Different modalities were used for identify the organisms.

The present study was done on 75 cases present with upper gastrointestinal symptoms. Endoscopy findings were recorded. Our study showed male predominance with a peak age group in the third to fourth decade. IHC is one of the gold standard method to detect H.pylori. We compare the two special stains Giemsa and Toludine blue in detection of H.pylori. Confirmatory was done by IHC.

- H.pylori infection shows slightly male predominance.
- Mostly diagnosed in 3rd and 5th decade with a mean age group of 43 years in this study.
- Out of 75 cases, H.pylori positivity seen in 53 cases (76%), which is more common in developing countries.
- Most of the patients were from low socioeconomic status.
- Most common endoscopic clinical findings was gastritis (63%).

- As we compare the two special stains with IHC, Toludine blue showed sensitivity of 88.1% which is close to sensitivity of Giemsa stain that is 88.6% in detection of H.pylori. false positivity was less than giemsa.
- Toludine blue is a simple procedure and does not requires acid differentiation step, cheaper than giemsa and more economical.
- Most of the mild colonization(40%) of bacilli were detected by IHC. So IHC is the most reliable one to identify the mild H.pylori intensity, modified coccoid form, single bacilli, and deeply positioned bacilli, which is not amenable by histochemical stains.
- According to revised Sydney system H.pylori gastritis were graded into mild, moderate and severe. Almost all grading of H.pylori infections associated with mononuclear inflammatory infiltrate, neutrophilic activity and mild degree of intestinal metaplasia.

In our study severe H.pylori colonization showed 60% of mild glandular atrophy. Other significant histopatholgical changes associated with H.pylori infection were lymphoid follicles, aggregates, epithelial degeneration, focal regeneration and villiform transformation.

VI. CONCLUSION

Chronic H.pylori infection has been identified as the important cause of chronic gastritis, gastric carcinoma and gastric lymphoma which is highly prevalent in developing countries like India and particularly in people with low-socioeconomic status leads to increased morbidity and mortality.Hence, the need of the hour is to device a low cost, easily feasible Laboratory test to identify H.pylori in initial stages of infection. Early treatment of H.pylori leads to essentially, prevention of malignancy like gastric carcinoma and lymphoma.In this study it has been compared the Giemsa and Toludine blue staining of gastric histopathological specimens for identification of H.pylori with the gold standard albeit costly investigation like, IHC since they both got good sensitivity and specificity and is a very valuable test for identification and early treatment of H.pylori in Primary Health centre and District Hospitals, where IHC technique is not feasible. It has concluded that Toludine blue staining is more economical, cheaper and technique easy to perform than Giemsa stain. Toludine blue staining remains a valuable study for identification of H.pylori in resource-poor settings and in a developing country like India.

REFERENCES

[1]. Marcela S. Boldt; Rivelle D. Pereira; Alfredo J. A. Barbosa, Histological identification of H. pyloristained by hematoxylin-eosin and Giemsa: review for quality control, J Bras Patol Med Lab, v. 51, n. 2, p. 108-112, April 2015.

[2]. Dussadee Sakonlaya, Anucha Apisarnthanarak, Nobutaka Yamada, Prakitpunthu Tomtitchong, Modified Toluidine Blue: an Alternative Stain for Helicobacterpylori Detection in Routine Diagnostic Use and Post-eradicationConfirmation for Gastric Cancer Prevention, Asian Pacific Journal of Cancer Prevention, Vol 15, 2014, pp.6983-6987.

[3]. Dunn BE, Cohen H, Blaser MJ. Helicobacter pylori. Clin Microbiol Rev.1997;10(4):720-41.

[4]. Koumi A, Filippidis T, Leontara V, Makri L, Panos MZ. Detection of Helicobacter pylori: A faster urease test can save resources. World JGastroenterology.2011 Jan;17(3):349-53.

[5]. Smith SI, Fowora MA, Otegbayo JA, Abdulkareem FB, Omonigbehin EA, Adegboyega A et al. Comparison of PCR with other diagnostic techniques for the detection of H. pyloriinfection in patients presenting with gastroduodenal symptoms in Nigeria. Int J MolEpidemiol Genet.2011 May; 2(2):178-84.

[6]. Ozturk S, Serinsoz E, Kuzu I et al. The Sydney System in the assessment of gastritis:Inter-observer agreement. The Turkish Journal of Gastroenterology 2001;12:36-9.

[7]. Paniagua GL, Monroy E, et al. Frequency of vacA, cagA and babA2virulence markers in Helicobacter pylori strains isolated from Mexican patients with chronic gastritis. Ann Clin MicrobiolAntimicrob.2009 Apr;30:8-14.

[8]. Pacifico L, Anania C, Osborn JF, Ferraro F, Chiesa C. Consequences of Helicobacter pyloriinfection inchildren. World J Gastroenterol.2010 Nov;16(41):5181-94.

[9]. Gold BD, Colletti RB, Abbott M, Czinn SJ, Elitsur Y,Hassall E et al. Helicobacter pylori infection in children: recommendations for diagnosis and treatment. J Pediatr Gastroenterol Nutr.2000 Nov;31:490-7.

[10]. Talebkhan Y, Mohammadi M, Khalili G, Sheykholeslami A, Rakhshani N, Mahboudi F et al. Detection of Helicobacter pyloriinfection by imported IgG ELISA kits

in comparison with Iranian homemade kit. Govaresh.2006;11(2):120-5.
[11]. Doosti A, ghasemi-dehkordi P. Helicobacter pylori vacAgenotypes in Shahrekordian (Iran) H. pylori positive patients. Res J Bio Sci.2009;4(1):11-5.
[12]. Glupczynski Y. Microbiological and serological diagnostic tests for Helicobacter pylori: an overview. BrMed Bull.1998;54(1):175-86.
[13]. Micu G, Staniceanu F, Zurac S, Popp C, Bastian A, Gramada E et al. Diagnosisand treatment in Helicobacter pyloriinfection. Rom J Itern Med.2010;48(3):239-47.
[14]. Chey WD, Wong BCY. American College of Gastroenterology Guideline on the Management of Helicobacter pyloriInfection. Am J Gastroenterol.2007 Aug;102(8):1808-25.
[15]. F. Kacar, N. culhaci et al, Histologic demonstration of Helicobacter pylori in gastric biopsies: Which is the best staining method? The Internet Journal of Pathology,2004; 3 (1): 310 – 5
[16]. Raziye Tajalli, Maliheh Nobakht et al, The Immunohistochemistry and Toludine blue – Roles for Helicobacter pylori detection in patients with gastritis. Iranian Biomed journal, 2013; 17(1): 36 – 41.
[17]. Fiaz Ahmad et al, Helicobacter pylori detection in chronic gastritis: A comparison of staining methods. J Ayub Med Coll Abbottabad 2011;23 (2), 112 – 114.
[18]. Pity IS. Identification of Helicobacter pyloriin gastric biopsies of patients with chronic gastritis: histopathological and immunohistochemical study.
[19]. Theeraya Simawaranon Bartpho, Wareeporn Wattanawongdon, Taweesak Tongtawee, Chatchanok Paoin, Kokiet Kangwantas, Chavaboon Dechsukhum, "Precancerous Gastric Lesions with Helicobacter pylori vacA+/babA2+/oipA+ Genotype Increase the Risk of Gastric Cancer", BioMed Research International, vol. 2020, Article ID 7243029, 8 pages, 2020
[20]. N. Bagheri, A. Razavi, B. Pourgheysari et al., "Up-regulated Th17 cell function is associated with increased peptic ulcer disease in Helicobacter pylori-infection," Infection, Genetics and Evolution, vol. 60, pp. 117–125, 2018.
[21]. Y. Saeidi, A. Pournajaf, M. Gholami et al., "Determination of Helicobacter pylori virulence-associated genes in duodenal ulcer and gastric biopsies," Medical Journal of the Islamic Republic of Iran, vol. 31, pp. 555–559, 2017.