International Journal of Research in Medical Sciences
Rajan A et al. Int J Res Med Sci. 2017 May;5(5):1851-1857
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20171599

Original Research Article

Correlation of serology with morphological changes in gastric biopsy of *H. pylori* infection

Amit Rajan[1], Prosenjit Ganguli[2]*, Niloy Pathak[2], Amar Ranjan[3], Ragini Thapa[4], Snehlata Tripathi[5]

[1]Department of Pathology, 160 Military Hospital, Masimpur, Silchar, Assam, India
[2]Department of Pathology, Command Hospital Kolkata, India
[3]Department of Lab Oncology, AIIMS New Delhi, India
[4]Department of Pathology, Military Hospital, Shillong, India
[5]Department of Pathology, Hind Medical College, Lucknow, Uttar Pradesh, India

Received: 04 April 2017
Accepted: 18 April 2017

***Correspondence:**
Dr. Prosenjit Ganguli,
E-mail: prosenjitganguli@gmail.com

ABSTRACT

Background: *Helicobacter pylori* is involved in many gastrodeudonal complications and many diagnostic tests are available for its identification. The present study was done with the objective to evaluate the morphological changes induced by *H. pylori* in the gastric mucosa and to correlate them with the severity of the infection.
Methods: This study was conducted in a tertiary care hospital from July 2013 to June 2014. 60 patients with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy were included in the study. Upper gastrointestinal endoscopy was performed on all patients. Hematoxylin and Eosin staining (H and E), modified Giemsa staining were performed on tissue sections and examined microscopically for gastritis and presence and absence of *H. pylori.*
Results: Out of 60 patients, 33 were male and 27 were females. Serology by immunochromatography technique was positive in 41 patients. Serology was found to have a sensitivity and specificity of 90.90% and 59.25% respectively. *H. pylori* was positive in 28 cases on H and E. With a sensitivity and specificity of 84.84% and 100% respectively. *H. pylori* was positive in 33 cases on modified Giemsa with a sensitivity and specificity of 100%.
Conclusions: Simultaneous morphologic and serological detection of H. pylori helps in its complete distribution and identification of its precancerous morphological nature.

Keywords: Helicobacter pylori, Hematoxylin and eosin staining, Modified Giemsa staining, Serology

INTRODUCTION

Helicobacter pylori is a spiral gram negative bacterium which was discovered by Marshall BJ and Warren R in 1982.[1] Studies have indicated the presence of *H. pylori* association with a variety of gastrointestinal disease including gastritis, duodenal and gastric non ulcer dyspepsia, duodenal and gastric ulcer, gastric adenocarcinoma and lyhmphoma.[2-4] The organism is present in gastric biopsy specimens of almost all patients with duodenal ulcer as well as most individuals with gastric ulcers.[5]The removal of organism by antimicrobial therapy is correlated with the resolution of symptoms and cure of diseases.[6]

The tests available for the diagnosis of *H. pylori* infection can be broadly divided into two types: invasive and non-invasive. Non-invasive tests include serological diagnosis, urea breath test, and stool antigen test. *H. pylori* specific antibodies have been detected in the

serum, saliva and urine.[7,8]Antibodies to *H. pylori* including IgG, IgA, IgM class is increased in the sera of patients harbouring *H. pylori* infection, as determined by ELISA/ ELFA/immunochromatographytechnique.[9-12]

Invasive tests require an endoscopic gastric biopsy specimen and include rapid urease test, histological examination and culture of biopsy.

H. pylori can be seen in routine hematoxylin and Eosin staining, but many newer staining methods have been devised for better visualization of *H. pylori*, including immunohistochemical stains.[13,14]

The present study attempted to document the morphologic changes in the gastric mucosa induced by *H. pylori* colonization and correlate them with the severity of the infection. The study has compared different diagnostic tests used for *H. pylori* detection.

METHODS

This study was conducted in the Department of Pathology, in a tertiary care hospital from July 2013 to June 2014. The study population comprised of all patients of either gender of any age with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy. 60 patients were included in the study. Patients who had received antibiotics, proton pump inhibitors, H_2 blockers or colloidal bismuth sulphate within the previous two months of endoscopy, patients with a history of gastric resection/vagotomy, patients with complicated peptic ulcer disease (haemorrhage/perforation) were excluded.

A blood sample of all patients selected for endoscopy was taken by venepuncture and serum separated and stored at -20^{o}C for *H. pylori* serology to detect IgG and/ or IgM antibodies by Immunochromatography technique. Upper gastrointestinal endoscopy was performed on all patients. Antral/ Pyloric biopsy were taken in each patient. The endoscope and biopsy forceps were disinfected in 2% gluteraldehyde solution before and after each case. Routine Hematoxylin and Eosin staining, modified Giemsa staining were performed on tissue sections of each case and then mounted with DPX.

H and E stained sections for all cases were examined microscopically for gastritis and presence and absence of *H. pylori*. These were also graded according to the updated Sydney system using the visual analog scale.[15]

RESULTS

A total of 60 cases fulfilled selection criteria, were selected for the study. Detailed history and examination findings were recorded in a predesigned proforma.

The cases belonged to any age group having gastric symptoms were distributed to different age groups. The maximum numbers of cases were in the age group of 41 to 60 years (45%). The cases in the age group 21-40 and 61-80 years were 40% and 13.3% respectively. There was 1 case in the age group of below 20 years.

The most common symptom encountered was epigastric pain which was seen in 44 (73.7%) cases, as shown in Figure 1. This was followed by either nausea or vomiting or both, which was seen in 23 (38.3%) cases. Bloating, belching and acid eructation was seen in 19 cases. Other uncommon symptoms seen were dyspepsia (4 cases), dysphagia, haematmesis and weight loss (2 cases) diarrhea, dysphagia along with dyspepsia, hiccups, loss of appetite, post prandial fullness (1 case each). Past history of tuberculosis and renal stone, cholelithiasis was present in 2 cases each. One case had a history of hernia.

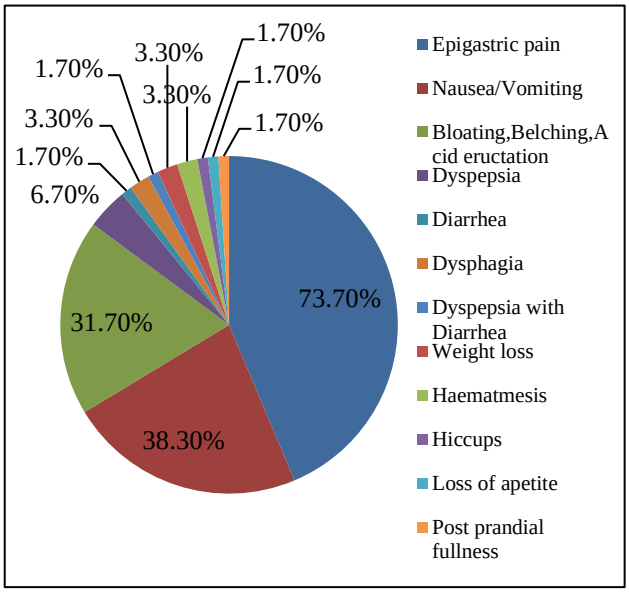

Figure 1: Symptom profile.

The most common symptom encountered in both male and female cases were epigastric pain- 22 males and also 22 females presented with epigastric pain. Nausea and vomiting was more common in male cases. 13male cases had history of nausea/vomiting, whereas 10 female cases complained of the same. Weight loss was seen only in two cases both were male.

Endoscopy findings have been summarised in Figure 2.55% cases were endoscopically within normal limits. 21.7% cases had mild antral gastritis, 6.7% cases showed erosive gastritis, duodenal ulcer and hyperaemia in the body of stomach were seen in 3.3% cases.

Severe antral gastritis, hyperaemia of proximal antrum, GERD with antral gastritis and Grade-I oesophageal varices with chronic antral gastritis, were seen in one case each.

All cases were subjected to serology test by immunochromatography technique, histological examination by haematoxylin and Eosin and modified

Giemsa. Serology by immunochromatography technique was done in all cases and was positive in 41 cases. Out of 60 cases 33 cases were positive for *H. pylori* on any one or more of the two tests. Out of these, 28 had visible *H. pylori* on H and E, 33 had visible *H. pylori* on modified Giemsa.

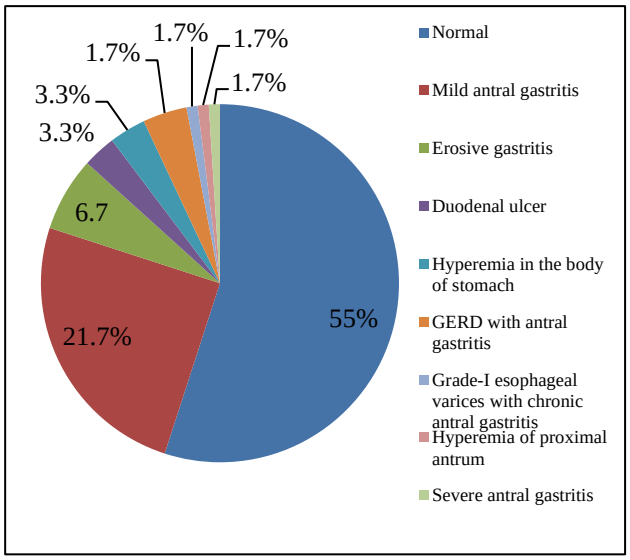

Figure 2: Endoscopic findings.

A total of 60 cases showed gastric inflammation on histology. Out of these 60 cases, 57 showed chronic inflammation, while 03 showed solitary acute inflammation.31cases showed activity (presence of neutrophils). Chronic atrophic gastritis was found in 39 cases. There was no case of gastric atrophy.

All H and E stained sections were graded for gastritis using some help from the visual analogue scale (VAS) of the updated Sydney system.

Six cases with chronic inflammation also showed presence of lymphoid aggregates. Glandular atrophy was seen in 39 cases out of which 35 had mild and 3 had moderate and 1 had severe glandular atrophy. The case with moderate atrophy had more positive *H. pylori*. Intestinal metaplasia was seen in three cases, both being mild. All showed presence of *H. pylori* with a positive serology.

As observed in Table 1, H and E was positive for *H. pylori* in 28 cases (84.84%) out of 33 cases which were positive for *H. pylori*. Modified Giemsa detected an additional 5 cases and increased the diagnostic yield by a further 15.15%. Serology performed by immunochromatography showed positive for 41 cases.

Table 1: Comparison of modified Giemsa, H and E and serology.

Test		*H. pylori*		Sensitivity	Specificity	PPV	NPV
		Positive	Negative				
H and E	Positive	28	0	84.84%	100%	100%	84.3%
	Negative	5	27				
MGG	Positive	33	0	100%	100%	100%	100%
	Negative	0	27				
Serology	Positive	30	11	90.90%	59.25%	73.17%	82.21%
	Negative	3	16				

Table 2: Correlation *H. pyloriinfection* with age and sex.

	Serology		*H. pylori*		Total
	Positive	Negative	Positive	Negative	
Age group (years)					
<20	0	1	0	1	1
21-40	17	7	16	8	24
41-60	20	7	13	14	27
61-80	4	4	4	4	8
Total	41	19	33	27	60
Gender					
Male	20	13	19	14	33
Female	21	6	14	13	27
Total	41	19	33	27	60

Table 2 shows maximum number of cases belonged to the age group of 41 to 60 years, while age group 21-40 showed maximum percentage of *H. pylori* positivity (66.66%). Out of 33 male cases 19 (57.57%) were positive for *H. pylori* and out of 27 female cases 14 (51.85%) were positive for *H. pylori* on histomorphology. Similarly, the seroprevalence of *H. pylori* infection was 48.78% (20 cases) in males and 51.21% (21 cases) in females.

Table 3: Correlation of *H. pyloriserology* with H and E and modified Giemsa.

Serology	*H. pylori* on H and E		H. pylori on modified Giemsa		Total
	Positive	Negative	Positive	Negative	
Positive	26	15	30	11	41
Negative	2	17	3	16	19
Total	28	32	33	27	60

Serology by immunochromatography technique correlated significantly with the presence of bacteria on histology (H and E, modified Giemsa). Table 3 shows that out of 41 cases positive by serology, 26 showed visible *H. pylori* on H and E stained sections while out of 19 cases which were negative for serology, only 2 showed *H. pylori* on H and E (false negatives). A significant correlation was seen between serology and modified Giemsa. Out of 41 cases which tested positive on serology 30 showed visible *H. pylori* on Modified Giemsa while out of 19 cases which were negative on serology 3 showed *H. pylori* on Modified Giemsa (false negative).

Table 4: Correlation of H. pyloriandgastritis with serology.

Biopsy findings	Serology positive	Serology negative	Total
Gastritis+ H. pylori+	30	03	33
Gastritis- H. pylori-	00	00	00
Gastritis + H. pylori-	11	16	27
Gastritis - H. pylori+	00	00	00
Total	41	19	60

The presence of *H. pylori* and gastric inflammation was correlated with the serology as shown in Table 4. The biopsy findings correlate well with serology status as 30 (90.90%) out of 33 cases with both gastritis and positive *H. pylori* had serology positive.

Table 5: Correlation of *H. pyloristatus* with acute inflammation.

H. pylori	Acute inflammation		Total
	Y	N	
Positive	0	33	33
Negative	3	24	27
Total	3	57	60

Acute inflammation alone did not correlate significantly with *H. pylori* status, as shown in Table 5. There was no case with positive *H. pylori* which showed acute inflammation. All the three cases which showed acute inflammation were negative for *H. pylori*.

Table 6: Correlation of *H. pyloristatus* with chronic inflammation.

H. pylori	Chronic Inflammation		Total
	Y	N	
Positive	33	0	33
Negative	24	3	27
Total	57	3	60

Table 7: Comparision of presence of lymphoid aggregates with *H. pyloristatus.*

H. pylori	Lymphoid aggregates		Total
	Present	Absent	
Positive	3	30	33
Negative	3	24	27
Total	6	54	60

Chronic inflammation score correlated significantly with *H. Pylori* status, as shown in Table 6. There was no case

in which chronic inflammation was absent but positive for *H. pylori*.

As shown in Table 7, 6 cases with chronic inflammation showed presence of lymphoid aggregates. Out of these, 3 (50%) showed the presence of *H. pylori*.

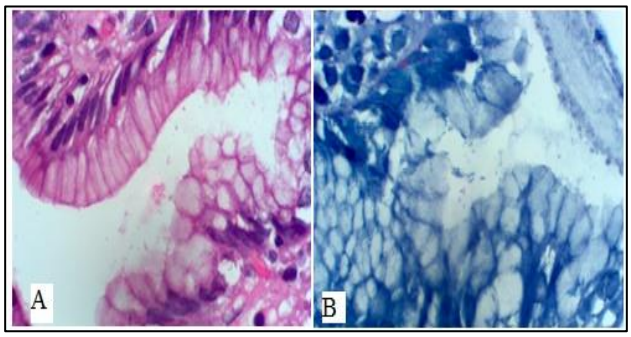

Figure 3: Numerous *H. pylori* in the lumen of gland. (A) H and E staining, 40 X; (B) Giemsa stain, 100 X.

DISCUSSION

There are few studies which have correlated serologic detection of *H. pylori* with morphological changes produced by it in the gastric mucosa. The present study attempted to document the morphologic changes in the gastric mucosa induced by *H. pylori* colonisation and correlate them with serology and the severity of the infection. The study also compared different diagnostic tests and evaluated the different staining methods used for *H. pylori* detection.

The present study comprised of 60 patients of either gender of any age, presenting with symptoms of dyspepsia and requiring an upper gastrointestinal endoscopy. The maximum number of patient was in the age group 41 to 60 years (45%) with the mean age being 45.4 years. Out of total 60 cases, 33 (55%) were males and 27 (45%) were females.

The most common symptom encountered in both male and female patients was epigastric pain which was seen in 22 (50%) males and 22 (50%) females. This was followed by either nausea or vomiting or both, which was seen more commonly in male patients presented in 13 (39.4%) as compared to that in female patients of which only 10 (37%) had this symptom.

In the present study, no abnormality was detected in most of the patients on endoscopic examination.

The seroprevalence of *H. pylori* infection in the present study was 68.33%, which was similar with the previous reports of Kate et al in his two studies done on cases with non-ulcer dyspepsia was 68% and 74.4%.[15,16]

In the present study, there was a slight preponderance of *H. pylori* infection in males (57.6%) as compared to females (42.42%). Most other studies have also reported a slight male preponderance of *H. pylori* infection.[17]

In this study, serology by immunochromatography technique to detect antibodies against *H. pylori* correlated significantly with presence of *H. pylori* on histology (H and E, modified Giemsa). Out of 41 cases positive by serology, 26 (63.41%) showed visible *H. pylori* on H and E, and 30(73.17%) showed on modified Giemsa stain.

Similar results were found by Booth et al who found a very low *H. pylori* positivity in cases with low IgG and IgA.[18] Perez-Perez et al also found that 33 out of 46 patients (71.7%) who were positive on serology showed *H. pylori*.[19] Similar results were found by Urita et al.[20]

In the present study, the sensitivity, specificity, PPV and NPV of serology was 90.90%, 59.25%, 73.17% and 82.21% respectively. These results were comparable to those of Urita et al.[20] She et al however, used stool antigen test as the gold standard and found lower values for specificity and PPV.[21] All the mentioned studies used ELISA technique for serology, whereas in the present study immuno chromatography technique was used.

There were 11 cases in the present study which were negative for *H. pylori* on histomorphology but had a positive serology. There can be several explanations for this. It may occur in chronic atrophic gastritis, a common condition in the evolution of *H. pylori* gastritis. In these patients, the gastric cavity becomes hostile to *H. pylori*, the organism disappears from the mucosa but antibody titres persist in the range of diagnostic titres for *H. pylori* infection.[9] There was 1 such cases in the present study which showed positive serology and glandular atrophy on morphology without the presence of *H. pylori*. Similar findings have also been reported in patients with chemical gastritis, particularly in patients treated with non-steroidal anti-inflammatory drugs (NSAIDs), who have a low prevalence of *H. pylori.*[9] Alternatively the possibility exists that the active site of infection was missed by biopsy specimens of the highly patchy distribution of *H. pylori* in the gastric mucosa.

In the present study, the cases which were positive for *H. pylori* on serology had gastritis on morphology. Similarly, Perez-Perez et al also found positive correlation between serology and gastritis on morphology in 88.6% cases.[19] Jones et al so found that out of 35 cases of gastritis, 29(83.9%) showed their presence of complement fixing antibodies.[22]

In the present study, there was a significant correlation between the severity of gastritis and the presence of *H. pylori* infection on histology. Similar results were seen by Tokunaga et al.[23]

In the present study, a total of six cases (10.0%) showed the presence of lymphoid follicles in addition to chronic inflammation. However, the presence of lymphoid

follicles did not correlate significantly with the presence of *H. pylori* as only 3 out of these 6 patients were positive for *H. pylori*. Similar results were seen by Shafii et al, who, although found lymphoid follicles in 46% cases, did not find a significant difference in the presence of lymphoid follicles between *H. pylori* positive and *H. pylori* negative cases.[24]

In the present study, acute inflammation was seen in 3 cases. However, this finding did not have a correlation with the presence of *H. pylori* as none out of these 3 cases was positive for *H. pylori*. Malik et al also found acute inflammation in 20% of cases (30 cases) but unlike the present study, they found *H. pylori* in 67% cases (20 out of 30). Intestinal metaplasia was seen in 3cases (5%).[25] All cases showed presence of *H. pylori* with a positive serology.

CONCLUSION

A simple test like modified Giemsa should be used routinely for detection of *H. pylori* as it is economical. Simultaneous morphologic and serological detection of *H. Pylori* helps in its complete distribution and identification of its precancerous morphological nature. The observations of the present study concluded that a significant correlation was existed between presence of *H. pylori* and severity of gastritis and also between serology and status of *H. pylori*

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet. 1984;1:1311-5.
2. Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J Infect Dis. 1990;161:626-33.
3. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori associated gastritis and primary B-cell lymphoma. Lancet. 1991;338:1175-6.
4. Graham DY, Klein PD, Evans DG, Fiedorek SC, Evans DJ Jr, Adam E, et al. Helicobcter pylori: Epidemiology,relationship to gastric cancer and the role of infants in transmission. Eur J Gatroenterol Hepatol. 1992(l);4:1-6.
5. Kumar V, Abul AK, Jon AC. Robbins and Cotran Pathologic basis of disease. 9th edition. New Delhi: Elsevier; 2014.
6. Kumar M, Yachha SK, Aggarwal R, Shukla S, Pandey R, Prasad KN, et al. Healing of chronic antral gastritis: effect of sucralfate and colloidal bismuth subcitrate. Ind J Gastroenterol. 1996;15(3):90-3.
7. Wu IC, Wu DC, Lu CY, Kuo CH, Su YC, Yu FJ, et al. Comparision of serum and urine ELISA methods for the diagnosis of Helicobacter pylori: a prospective pilot study. Hepatogastroenterol. 2004;51:1736-41.
8. Luzza F, Maletta M, Imeneo M, Marcheggiano A, Iannoni C, Biancone L, et al. Salivary specific immunoglobulins G in the diagnosis of Helicobacter pylori infection in dyspeptic patients. Am J Gastro. 1995;90(10):1820-5.
9. Martin-de-Argila C, Boixeda D, Canton R, Valdezate S, Mir N, De Rafeal L, et al. Usefulness of the combined IgG and IgA antibody determinations for serodiagnosis of Helicobacer pylori infection. Eur J Gastroenterol Hepatol. 1997;9(12):1191-6.
10. Thijs BC, van Zwet AA, Thijs WJ, Oey HB, Karrenbeld A, Stellaard F, et al. Diagnostic tests for Helicobacter pylori: a prospective evaluation of their accuracy, without selecting as the gold standard. Am J Gastroenterol. 1996;91:2125-9.
11. Graham DU, Evans DJ, Peacock J, Baker JT, Schrier WH. Comparison of rapid serological tests (FlexSure HP and Quickvue) with conventional ELISA for detection of *H.pylori* infection. Am J Gastroenterol. 1996;91:942-8.
12. Wilcox MH, Dent TH, Hunter JO, Gray JJ, Brown DF, Wight DG, et al. Accuracy of serology for the diagnosis of Helicobacter pylori infection; a comparison of eight kits. J Clin Pathol 1996;49:373-6.
13. Laine L, Lewin DN, Naritoku W, Cohen H. Prospective comparision of H and E, Giemsa and Genta stains for the diagnosis of Helicobacter pylori. Gastroint Endosc. 1997;45(6):463-7.
14. Genta RM, Robason GO, Graham DY. Simultaneous visualization of Helicobacter pylori and gastric morphology: a new stain. Hum Path. 1994;25:221-6.
15. Gill HH, Desai HG, Majumdar P, Mehta PR, Prabhu SR. Epidemiology of Helicobacter pylori: the Indian scenario. Ind J Gastroenterol. 1993;12(1):9-11.
16. Gill HH, Majumdar P, Shankaran K, Desai HG. Age related prevalence of Helicobacer pylori in Indian subjects. Ind J Gastroenterol. 1994;13:92-4.
17. Kang G, Rajan DP, Patra S, Chacko A, Mathan MM. Use of serology, the urease test and histology in diagnosis of Helicobacter pylori infection in symptomatic and asymptomatic Indians. Ind J Med Res. 1999;110:86-90.
18. Booth L, Holdstock G, MacBride H, Hawtin P, Gibson JR, Ireland A, et al. Clinical importance of Campylobacter pylorides and associated serum IgG and IgA antibody responses in patients undergoing upper gastrointestinal endoscopy. J Clin Pathol. 1986;39:215-9.
19. Perez-Perez GI, Brown WR, Cover TL, Dunn BE, Cao P, Martin JB. Correlation between serological and mucosal inflammatory responses to

Helicobacter pylori. Clin Dia Lab Immun. 1994;1:325-9.
20. Urita Y, Hike K, Torii N, Kikuchi Y, Kurakata H, Kanda E, et al. Comparison of serum IgA and IgG antibodies for detecting Helicobacter pylori infection. Int Med. 2004;43:548-52.
21. She RC, Wilson AR, Litwin CM. Evaluation of Helicobacter pylori IgG, IgA and IgM serologic testing compared stool antigen testing. ClinVacc Immun. 2009;16:1253-5.
22. Jones DM, Lessells AM, Eldridge J. Campylobacter - like organisms on the gastric mucosa: culture, histological and serological studies. J clinpathol. 1984;37:1002-6.
23. Tokunaga Y, Shirahase H, Yamamoto E, Inao R, Hamaguchi S, Kanaji K, et al. Modified rapid urease test for H. pylori detection in relation to an immunohistochemical stain. J Gastroenterol Hepatol. 2000;15:617-21.
24. Shafii M, Nikzad SE, Kasiri H, Naghipour M. Histopathological evaluation of chronic gastritis with and without Helicobacter pylori colonization: a study from Iran. Malaysian J Pathol. 2008;30:27-30.
25. Malik GM, Mubarik M, Kadla SA. Helicobacter pylori infection in endoscopy biopsy specimens of gastric antrum: Laboratory diagnosis and comparative efficacy of three diagnostic tests. Diag Therp Endoscop J. 1999;6:25-9.

Cite this article as: Rajan A, Ganguli P, Pathak N, Ranjan A, Thapa R, Tripathi S. Correlation of serology with morphological changes in gastric biopsy of *H. pylori* infection. Int J Res Med Sci 2017;5:1851-7.