Evaluation of Antral Gastric Biopsies. A Study of 50 Patients at Mayo Hospital

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Fifty patients presenting with dyspeptic symptoms at Mayo Hospital during five month period (October 2009 to February 2010) underwent endoscopic examination. Biopsies were taken from gastric antral mucosa and sent to Pathology Department of KEMU. Helicobacter Pylori infection was diagnosed in 24 of these biopsies. There were 13 males and 11 females and their ages ranged between 18-80 years. The most common presenting complaint of these patients was heart burn and upper abdominal pain. Histologically 92% of biopsies showed chronic inflammation, 18% showed neutrophilic infiltration, 10% showed Intestinal metaplasia and 2% had atrophy of glands. Dysplasia was noted in 2% and Adenocarcinoma was diagnosed in 4% of biopsies. Our Population has higher percentage of infection and premalignant lesions as compared to developed nation.

Keywords: Dyspepsia, Helicobacter Pylori, Premalignant lesions.

Introduction

Awareness about chronic gastritis is very important as it is a distinct clinical entity and is an initial step leading to the peptic ulcer and cancer. Therefore, a detailed classification system including morphological, pathophysiological and etiological factors, as well as the clinical basis is mandatory in the evaluation of patients with chronic gastritis.[1] Chronic gastritis is still a confusing terminology; however it has improved after the development of modern investigative techniques in clinical and laboratory practice. In 1970' and later, more data was documented on the role of auto-antibodies against parietal cells and intrinsic factors in diffuse gastritis in cases of pernicious anemia. These auto-antibodies are directly proportional to severity of gastritis, increased atrophy, apoptosis in gastric mucosa, and histopathological features that are same as those of autoimmune gastritis (AIG).[2]

Several classifications systems have been introduced with the goal of understanding the status of the gastric mucosa. Before the rediscovery of Helicobacter pylori, it was believed that different patterns of gastritis were associated with different diseases.[3] In 1973, Strickland and Mackay suggested classifying patients as having type A gastritis if they showed inflammatory and atrophic changes in the body along with anti-parietal cell and intrinsic factor antibodies.[4] Other patients were classified as type B gastritis. Further studies stressed that type B gastritis was present in different grades of intensity in the antrum and body of stomach, suggesting that type B gastritis may progress proxymally in stomach.[4]

The present classification of chronic gastritis the Updated Sydney System – takes into account the topography and morphology of gastric mucosal changes along with microbiological aspects of the disease.[5] In many studies, cultures have confirmed the significant role of H.pylori in chronic gastritis and duodenal ulcer (DU) disease.[6] Although antral predominant gastritis is the prevalent pattern of gastritis in duodenal ulcer, intestinal metaplasia in the gastric corpus may be found with geographic differences.[7] These findings suggest that duodenal ulcer and gastric cancer are not different diseases but are rather ends of the spectrum of H. pylori infection. It is now suspected that duodenal ulcer and gastric cancer are two ends of one disease, namely, H. pylori infection.[8]

The major landmark in the recent history of gastritis was the discovery of Helicobacter pylori as the cause for approximately 90% of cases of chronic gastritis. Later, the classification of gastritis was rather simplified from many conflicting nomenclatures to one, the Sydney System [9]. The updated Sydney System of chronic gastritis classification is now widely being used because it has taken into account the topography, morphology and microbiological aspects of the disease.[10] The existence of bacteria colonizing the gastric mucosa has been recognized for a long time.[11] In the last years there have been numerous publications revealing the role of HP (Helicobacter Pylori) in the pathogenesis of gastric carcinomas, gastric M.A.L.T. lymphomas and peptic ulcer disease.[12] HP is one of the most prevalent infections in the world. The incidence of HP in healthy individuals in their 3rd decades is 10% while the incidence of HP rises to over 60% among people in their 60's.[12]

The aim of this study was to evaluate the role of endoscopic biopsies for detection of Helicobacter Pylori to see the frequency of this infection and histological changes that are strongly associated with it, in our setup.

Materials and Methods

Fifty (50) endoscopic antral biopsies were obtained from patients who had undergone evaluations for dyspepsia, during 5 month period, from October 2009 to February 2010. Helicobacter Pylori (HP) status had been determined by histopathologic examination (H&E and modified Giemsa). Biopsy materials were fixed in 10% buffered formalin, embedded in paraffin and 4-5 µm sections were obtained. The sections were stained with Haematoxylin & Eosin

(H&E) and May Grünwald Giemsa for histopathologic examination. The histopathologic detection of Helicobacter pylori in gastric biopsy (H&E) specimens was considered the gold standard for the diagnosis of H pylori infection. When patients came to collect the report of histopathology, their clinical history was taken and their serum was collected to perform anti-H.pylori antibody (Ig G). Blood samples were taken only of patients who were positive for HP on histopathology.

The biopsies were scored semi-quantitatively by two histopathologists, according to the updated Sydney classification system (USCS). Variables that were analyzed included the presence or absence of HP, density of H pylori infection, the degree of chronic inflammation, inflammatory activity, atrophy, intestinal metaplasia, and surface epithelial damage (Dysplasia/Malignancy). Before grading biopsy specimens, both pathologists reached a consensus on the scoring of gastritis through interactive sessions using a multiheaded microscope. Subsequently all biopsy specimens were graded. Sections of Haematoxylin and Eosin were compared with Geimsa stain. Inter-observer variability was also analyzed using weighted kappa scores.

Chronic Gastritis

"Chronic gastritis" was defined as the presence of a uniform infiltration of the superficial and or deep lamina propria by lymphocytes and plasma cells.[13] "Activity "was defined as the additional presence of neutrophils.[14]

We used the Updated Sydney system (USCS) for grading the gastritis. The main aims of the USCS of gastritis were to improve uniformity in histopathological reporting and to provide a flexible matrix of rules for grading the histological features.[15]

The USCS has a scale of 0-3 for scoring the features of chronic gastritis. In order to improve assessment of minor degrees of alteration, a detailed histopathological classification was used, which also provided numerical data for statistical analysis. Each category (mild, moderate, and severe) was divided into two subcategories, resulting in a score on a scale of 0-6 (none: 0, mild: 1-2, moderate: 3-4, severe: 5-6) corresponding to the USCS as previously described.[16]

Density of Hpylori Colonisation

The density of H pylori colonization was graded as follows.[17]

None: 0

- Mild degree of H pylori colonization:
- 1: H.pylori found only in one place after a careful search.
- 2: only a few H pylori found.
- Moderate degree of H pylori colonization:
- 3: scattered H pylori found in separate areas/foci.
- 4: numerous H pylori in separate areas/foci.
- Severe degree of H pylori colonization:
- 5: nearly complete gastric surface covered by a layer of H pylori.
- 6: continuous gastric surface coverage by a thick layer of H pylori.

Atrophy

Atrophy was evaluated in the antral and corpus mucosa according to the USCS and semi quantitatively graded as focal, mild, moderate or severe atrophic gastritis. "Mucosal atrophy" was defined as a loss of specialized gastric glands (Parietal cells) in mucosa, partly replaced by intestinal metaplastic epithelium. It was characterized by architectural changes manifested by variation in the volume and irregularity in the shape, branching, and spacing of the glands. Other factors such as lamina propria, including myofibroblasts and inflammatory cells, might react with the gland to produce structural alterations, which may lead to architectural, metaplastic, proliferative and functional changes.

- None: 0.
- Mild degree of gastric atrophy i.e. up to 25 % atrophic gastric glands.
  - 1: foci where a few gastric glands were lost or replaced by intestinal-type epithelium.
  - 2: small areas in which gastric glands had disappeared or been replaced by intestinal-type epithelium.
- Moderate degree of gastric atrophy, i.e. up to 25-50 % atrophic gastric glands.
  - 3: upto 25% gastric glands lost or replaced by intestinal type epithelium.
  - 4: 25-50% of gastric glands lost or replaced by intestinal-type epithelium.
- Severe degree of gastric atrophy, i.e. > 50% atrophic gastric glands.
  - 5: more than 50% of gastric glands lost or replaced by intestinal-type epithelium.
  - 6: only a few small areas of gastric glands remaining.[10]

Degree of Intestinal Metaplasia

The degree of intestinal metaplasia was graded according to the amount of glandular tissue replaced by intestinal-type epithelium:

- 0: none.
- Mild degree of intestinal metaplasia i.e. upto 25 % gastric glands showing intestinal metaplasia.
  - 1: only one focus (one crypt) replaced by intestinal-type epithelium.
  - 2: one focal area (1-4 crypts) in one of two biopsies.
- Moderate degree of intestinal metaplasia i.e. upto 25-50% gastric glands showing intestinal metaplasia.
  - 3: two separate foci.
  - 4: multiple foci in one or both biopsies.
- Severe degree of intestinal metaplasia i.e. upto >50% gastric glands showing intestinal metaplasia.

- 5: more than 50% gastric epithelium diffusely replaced by intestinal metaplasia.
- 6: only a few small area of gastric epithelium are not replaced by intestinal metaplasia.

Degree of Inflammatory Activity

The degree of inflammatory activity was scored according to the density of neutrophils in the gastric mucosa:

- None: 0.
- Mild degree of activity:
- 1: only one crypt involved per biopsy.
- 2: two crypts involved per biopsy.
- Moderate degree of activity:
- 3: many crypts (up to 25%) involved per biopsy.
- 4: 25-50% of crypts involved per biopsy.
- Severe degree of activity:
- 5: more than 50% of crypts involved per biopsy.
- 6: all crypts involved.

Superficial Epithelial Damage

Superficial epithelial damage was scored as follows:

- None: 0.
- Mild degree of Superficial epithelial damage:
- 1: slight.
- 2: mild degeneration in the top of the epithelial cells.
- Moderate degree of superficial epithelial damage:
- 3: moderate degeneration with isorientation of the epithelial lining.
- 4: indistinct cell borders at the surface of the epithelium.
- Severe degree superficial epithelial damage:
- 5: flattened epithelial cells with severe degeneration and enlarged nuclei.
- 6: flattened to erosive epithelium of the entire surface.

Degree of Chronic Inflammatory Infiltrate

The degree of chronic inflammatory infiltrate in the gastric mucosa (lymphocytes, plasma cells) was scored as follows:

- None: 0.
- Mild degree of chronic inflammatory infiltrate:
- 1: scattered chronic inflammatory cells, less than 10 in each high power field.
- 2: scattered chronic inflammatory cells > 10 cells /high power field.
- Moderate degree of chronic inflammatory infiltrate:
- 3: some areas with dense chronic inflammatory cells.
- 4: diffuse infiltration with dense chronic inflammatory cells.
- Severe degree of chronic inflammatory infiltrate:
- 5: nearly the whole mucosa contains dense chronic inflammatory cells which separate the gastric glands.
- 6: entire mucosa contains a dense chronic inflammatory cell infiltrate.

Table 1: *Sex wise characteristic of different patients.*

Patients	Mean age	Number of HP (+) on Histology	H. Pylori antibody (IgG) positive	Gastric Atrophy	Activity	Intestinal metaplasia	Chronic inflammation	IEN	Gastric Ca
Male n=25	42.52 ± 18.57	13	12	01	03	01	26	01	01
Female n=25	32.96 ± 12.82	11	08	00	06	04	20	00	01
Total	37.74 ± 16.51	24	20	01	09	05	46	01	02

Key: HP: Helicobacter Pylori, IEN: Intraepithelial Neoplasia, Ca: Carcinoma

Table 2: *Presenting complaints of patients underwent antral gastric biopsy for Detection of H Pylori*.

Complaints	Abdominal Pain	Heart Burn	Bloating	Nausea / Vomiting	Dark stool	Fatigue
Positive symptoms	36 (72%)	41 (82%)	31 (62%)	20 (40%)	12 (24%)	24 (48%)
Negative symptoms	14 (28%)	9 (18%)	19 (38%)	30 (60%)	38 (76%)	26 (52%)

Table 3: *Gross finding of patients underwent antral gastric biopsy for Detection of H. Pylori.*

Gross findings	Hyperemia	Erosions	Ulcer	Nodularity	Growth	LLES
Positive findings	33	21	8	4	8	2
Negative findings	17	29	42	46	42	48
Total Patients	50	50	50	50	50	50

Key: LLES: Lax lower esophageal sphincter.

Results

The study included 50 patients attending for Endoscopy which included 25 males and 25 females. Mean age of males was 42.52±18.57 and females were 32.96 ± 12.82. Total 24 patients were positive for Helicobacter Pylori (HP) infection on histopathology (H&E) and 31 were positive on Geimsa stain. Out of 24, 13 were males and 11 were females. Twenty (20) of these 24 patients were positive for IgG antibody directed against H.Pylori in serum.

On analyzing the presenting complaints of patients, 82% (41/50) complained of heart burn, 72% (36/50) of upper abdominal pain, 62% (31) of bloating, 48% (24) of fatigue, 24% (12) of dark stool, and 20 (40%) of nausea and vomiting.

Endoscopic examination revealed that 66% of patients had hyperemia and redness in gastric mucosa, 42% had small erosions, 16% had ulcers, and 16% had a mass or polypoidal growths in stomach while 4% had lax lower esophageal sphincters.

Morphologically 92% (46) biopsies showed infiltration by chronic inflammatory cells, 18% (09) showed neutrophilic activity in glands and submucosal, 10% (05) had intestinal metaplasia, 4% (02) had Adenocarcinoma, 2% (01) had atrophy of glands, and 2% (01) had Dysplasia.

Another important finding was that 52% of males showed chronic inflammation associated with H.Pylori while 40% were females. Twelve % (12%) of female biopsies was positive for neutrophilic activity and 6% of male biopsies showed neutrophils. Gastric atrophy was noted in 2% of males only.

Table 4: *Comparison of Different modalities used for detection of H Pylori.*

H&E n = 24	Anti H.Pylori Antibody (Positive)		Total
Positive	20	4	24
Negative	0	4	4
	20	8	28

Sensitivity: 100%, Specificity: 50%, Accuracy: 85%, PPV: 84 %, NPV: 100%

Table 5: *Various lesions interpreted on Histopathology.*

Lesion	None	Mild	Mod	Severe	Total
Density of HP	26	09	15	0	50
Atrophy	49	01	0	0	50
Intestinal Metaplasia	45	05	0	0	50
Neutrophil activity	41	07	02	0	50
Epithelial damage	34	11	03	02	50
Chronic inflammation	04	22	22	2	50

Discussion

At least half of world's population is infected with H.pylori; making it the most widespread infection in the world[1]. Third world has much infection higher rates than the west, due to low socioeconomic status and poor hygienic levels.[18] In Pakistan, 90% of adults have been reported to be carrying HP infection[19]. HP is believed to be the main pathogen in the causing chronic gastritis, gastric carcinomas, gastric M.A.L.T. lymphomas and peptic ulcer disease.[12] HP infection was classified by the World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) in 1994 as a group-1 carcinogen in humans. This conclusion was based epidemiologically [20].However gastric pathologies are caused by multiple factors rather than HP being the only culprit. As we found out that half of the patients presenting with dyspeptic symptoms did not have HP infection.

In a Korean study, it was found that among population infected with HP, 70% were males and 30% were females. In our study we found that in our settings 52% males and 40% of females were infected with HP.[21] Average age of Korean subjects was 49.7 ± 10.4 years. However mean age of our patients' was 37.74 ± 16.51.

The sensitivity of the H&E stain is low in tracking HP, probably due to the lack of contrast between the bacteria and the surrounding tissues. The specificity of the H&E is also low due to its non-specific staining of the bacteria residents other than HP in the stomach.[16] We believe that H&E in combination with a special stain such as Geimsa for identifying the bacteria is rather more cost effective in demonstrating the bacteria. This was supported by the fact that with Geimsa stain we were able to find 31 cases to be positive for HP and on H&E we found 24 to be positive.

In past, some studies also related anemia and HP infection. However in our study we did not find any significant fall in hemoglobin levels in our patients. Average hemoglobin in our patients was 11.69 ± 1.52.

Patients presenting with dyspeptic symptoms most commonly showed hyperemia, redness and small erosions on endoscopic examination. Ulcers or growths were noted in a very small population. Endoscopic findings were not much helpful in assessing the grade of gastritis or presence of HP. Although as noted in previous studies, erosions, erythema and friability can be related to presence of inflammation and activity.[22]

We also compared the groups having HP and the group which did not have HP on histopathology. Degree of chronic inflammation was almost equal in both infected by HP and uninfected patients. Neutrophilic infiltration was more in HP infected patients (6 biopsies), while the uninfected group showed activity in 3 biopsies only.

Another study conducted in Spain showed Intestinal metaplasia in 5.6%, atrophy in 1.3% and Dysplasia in 0.2% of patients.[23] However, our study showed slightly higher values such as Intestinal metaplasia in 10%, atrophy in 2% and Dysplasia in 2% of patients. Metaplasia was noted more in HP infected group. This indicates that we should stress upon endoscopic biopsy examination in our population to diagnose premalignant lesions as early as possible.

Frequency of Dysplasia and malignancy was almost same in both HP positive and negative groups. This correlates to other studies showing that HP is usually absent in advanced premalignant lesions of stomach.[23]

Table 6: *Morphologic findings, gastric activity and H.pylori intensity in different patients.*

	Gastritis induced by H Pylori	**Gastritis without H Pylori**
Dysplasia	00	01
Carcinoma	01	01
Lymphoid Hyperplasia	00	03
Neutrophilic activity	06	03
Chronic gastritis		
Mild	11	11
Moderate	11	11
Severe	01	01
Density of HP		
Mild	09	Nil
Moderate	15	Nil
Severe	00	Nil

References

1. Marusawa H. Mechanisms of H.pylori infection induced gastric carcinogenesis. Gan To Kagaku Ryoho, 2010 Jan; 37 (1): 23-7.
2. Bergman, M. P., C. M. Vandenbroucke-Grauls, et al. "The story so far: Helicobacter pylori and gastric autoimmunity." Int Rev Immunol. 2005; 24 (1-2): 63-91.
3. Chlumska, A., L. Boudova, et al. Autoimmune gastritis. A clinicopathologic study of 25 cases. Cesk Patol. 2005; 41 (4): 137-42.
4. Strickland, R. G. and I. R. Mackay.A reappraisal of the nature and significance of chronic atrophic gastritis.Am J Dig Dis. 1973; 18 (5): 426-40.
5. Recavarren-Arce, S., R. Leon-Barua, et al.Helicobacter pylori and progressive gastric pathology that predisposes to gastric cancer. Scand J Gastroenterol Suppl. 1991; 181: 51-7.
6. Recavarren-Arce, S., R. Leon-Barua, et al.. "Helicobacter pylori--associated chronic gastritis in Peruvian adolescents is very common and severe. J Clin Gastroenterol. 1995; 20 (4): 335-7.
7. El-Zimaity, H. M. T., O. Gutierrez, et al.Geographic differences in the distribution of intestinal metaplasia in duodenal ulcer patients.Am J Gastroenterol. 2001; 96 (3): 666-72.
8. Buzas, G. M.History of the discovery of Helicobacter pylori. Orvostort Kozl.2004; 49 (3-4): 45-56.
9. Price, A. B.Classification of gastritis--yesterday, today and tomorrow.Verh Dtsch Ges Pathol. 1999; 83: 52-5.
10. Manxhuka-Kerliu, S. S. Telaku et al. Helicobacter pylori gastritis updated Sydney classification applied in our material. Prilozi. 2009; 30 (1): 45-60.
11. Vaira, D. P. Malfertheiner et al. Diagnosis of Helicobacter pylori infection with a new non-invasive antigen-based assay. HpSA European study group. Lancet. 1999; 354 (9172): 30-3.
12. Koshida, Y., W. Koizumi et al. Association of Helicobacter pylori-dependent gastritis with gastric carcinomas in young Japanese patients: histopathological comparison of diffuse and intestinal type cancer cases. Histopathology. 2000; 37 (2): 124-30.
13. Dooley, C. P., H. Cohen et al. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons.N Engl J Med. 1989; 321 (23): 1562-6.
14. Bayerdorffer, E., N. Lehn et al.Difference in expression of Helicobacter pylori gastritis in antrum and body. Gastroenterology. 1992; 102 (5): 1575-82.
15. Chen, X. Y., R. W. van der Hulst et al. Interobserver variation in the histopathological scoring of Helicobacter pylori related gastritis.J Clin Pathol. 1999; 52 (8): 612-5.
16. Aydin, O. R. Egilmez et al. Interobserver variation in histopathological assessment of Helicobacter pylori gastritis. World J Gastroenterol. 2003; 9 (10): 2232-5.

17. Chen, M. Y., D. J. Ott et al. Gastritis: classification, pathology, and radiology. South Med J .2001. 94 (2): 184-9.
18. Malaty HM. Epidemiology of Helicobacter pylori infection. Best Pract Res Clin Gastroenteral 2007; 21: 205-14.
19. Yakoob J, Jafri N, Jafri W. Polymerase chain reaction in the detection of Helicobacter pylori infection. J Coll Physicians Surg Pak 2004; 14: 153-6.
20. Blaser, M. J.Hypotheses on the pathogenesis and natural history of Helicobacter pylori-induced inflammation. Gastroenterology. 1992; 102 (2): 720-7.
21. Do M Y, Lee Y C, Choi C H et al. The changes in prevalence and the related factors of Helicobacter Pylori infection in Korean health check-up during 8 years. Korean J Gastroenteral. 2009 Feb; 53 (2): 76-83.
22. Ohkusa T, Kumagai J, Tanizawa T. Changes in endoscopic features and histological findings of H.pylori related gastritis with a follow-up over a year after eradication of H.pylori. 1999 Jan; 57 (1): 173-8.
23. Chacaltana A, Rodríguez C, Urday C, Ramon W, Espinoza J, et al. Preneoplastic gastric lesions and helicobacter pylori in endoscopic detection and early diagnosis of gastric cancer in a population of a medium and high socio-economic level. Rev Gastroenterol Peru. 2009; 29 (3): 218-25.