ORIGINAL ARTICLE

Histopathological Evaluation of *H. Pylori* Density and its Correlation with Activity, Atrophy and Intestinal Metaplasia

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ABSTRACT

Objective: The objective of this study was to determine the correlation of bacterial density with severity of the degree of neutrophilic infiltrate, atrophy and intestinal metaplasia after analyzing the density of *H. Pylori* semi quantitatively in chronic gastritis patients.

Study Design: The study was a cross sectional correlational study.

Place and Duration of Study: The study was conducted in Army Medical College Rawalpindi from 2nd December 2011 to 1st December 2012.

Materials and Methods: A total of one hundred gastric antral biopsies of *H. Pylori* associated chronic gastritis including all ages and both genders were included in the study. Most of the specimens that were received from the department of Gastroenterology Military Hospital Rawalpindi were fixed in 10% formaline. The tissue was processed in histopathology department. Giemsa stain was used for demonstration of *H. Pylori*. The density of *H. Pylori*, activity, atrophy and intestinal metaplasia were graded using a detailed histopathological classification. SPSS 17 was used to analyze the findings. Relationship between density of *H. Pylori* and other variables was calculated through Spearman's rank correlation test. The findings were considered to be statistically significant if p value was found to be less than 0.05 ($p<0.05$).

Results: An overall significant weak positive correlation was observed between grades of *H. Pylori* density and degree of neutrophilic activity ($r_{s=}$ 0.416). There was significant but weak relationship between grades of *H. Pylori* density and grades of atrophy ($r_{s=}$0.306). Intestinal metaplasia also revealed very weak association with grades of *H. Pylori* density ($r_{s=}$0.287).

Conclusion: In conclusion this study shows the semi quantitative determination of histological parameters and corroborates that, the greater the load of *H. Pylori* infection, the more is the degree of neutrophilic activity, atrophy and intestinal metaplasia.

Key Words: *Atrophy, Chronic Gastritis, Helicobacter Pylori, Intestinal Metaplasia.*

Introduction

Gastritis a clinical condition with upper abdominal discomfort is characterized by inflammation of the gastric mucosa and is the commonest condition observed in biopsies of stomach.[1] Gastritis is classified according to the underlying etiology i.e. *Helicobacter pylori* (*H. Pylori*), autoimmunity, bile reflux, allergic response, NSAIDs and the histopathologic pattern.[2] Infection with *H. Pylori* denotes a key factor in the etiology of several gastrointestinal ailments, ranging from chronic active gastritis to peptic ulceration, gastric MALT lymphoma and gastric adenocarcinoma.[3] Worldwide as a minimum 50% of people are infected, however an exact estimate is difficult, because precise data is unavailable from unindustrialized countries.[4] The *H. Pylori* gastritis prevalence is high in developing Asian countries.[5] Like all developing countries, Pakistan also has high *H. Pylori* prevalence. According to a research carried out in Islamabad Pakistan, 88 percent dyspeptic gastritis patients had *H. Pylori* infection.[6]

Almost half of this world's population is colonized with *H. Pylori*, and majority of infected individuals

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develop chronic inflammation.[7] In long duration disease, intestinal metaplasia and gastric atrophy are observed.[8] Conferring to the western data, intestinal metaplasia and atrophy are premalignant disorders seen in patients with *H. Pylori* chronic gastritis.[9] Simple gastritis may progress and can lead to atrophy, intestinal metaplasia, dysplasia and gastric carcinoma. The fundamental step is in these events is atrophy.[10] Hence, the role of *H. Pylori* in progression of gastric carcinoma seems to be the initiation of atrophy, indicating that research should focus on this event. Moreover, the bacterial density has been correlated with gastric inflammation.[11] A study was carried out at Army Medical College Rawalpindi showing that the density of *H. Pylori* in biopsy proven gastritis is positively correlated with chronic inflammatory infiltrate.[12]

The Sydney System was devised by a group of experts in Sydney, Australia in 1990. The new Updated Sydney System devised in Housten, Texas in 1994 uses none, mild, moderate, severe grades for the histopathological features of chronic gastritis.[13] A detailed histopathological classification can be used to improve assessment and avoid minor degrees of alteration. This study was designed to analyze the density of *H. Pylori* in patients of chronic gastritis and to address whether there was a correlation between bacterial density and severity of degree of neutrophilic infiltrate, atrophy and intestinal metaplasia.

The objectives of the current study were to determine the correlation of bacterial density with severity of the degree of neutrophilic infiltrate, atrophy and intestinal metaplasia after analyzing the density of *H. Pylori* semi quantitatively in chronic gastritis patients.

Materials and Methods

The present cross-sectional correlational study was carried out in the Pathology department of Army Medical College in collaboration with Military Hospital Rawalpindi, Pakistan from 2nd December 2011 to 1st December 2012. One hundred gastric antral biopsies of chronic gastritis patients of all ages and both sexes were included in the study. Gastric biopsies of patients who were receiving or had received *H. Pylori* eradication treatment were not included in the study. Most of the specimens were received from the department of Gastroenterology Military Hospital, Rawalpindi. The specimens were stored in 10% formal saline and were collected in Pathology Lab. Each sample was given a laboratory number.

The related clinical information was taken from laboratory. Data included age, sex, symptoms, history, concomitant medication (intake of antibiotics, proton pump inhibitors, and non-steroidal anti-inflammatory) and results of endoscopic investigations. The collected data was entered in already designed patient's proforma. The tissue was processed in histopathology laboratory and slides were stained with hematoxylin and eosin. Giemsa stain was used for demonstration of *H. Pylori*.

Gastric biopsies of 100 patients diagnosed as *H. Pylori* gastritis were included in the study. The Updated Sydney System uses a scale of 0-3 for scoring the histopathological features of chronic gastritis. We used a detailed histopathological classification in order to improve assessment and avoid minor degrees of alteration.[13] The following histopathological parameters were examined on each slide: density of *H. Pylori*, inflammatory activity, atrophy and intestinal metaplasia. Each category (mild, moderate, and severe) was further subdivided into two, resulting in a score on a scale of 0-6 (none, 0; mild, 1-2; moderate, 3-4; severe, 5-6).[14] This classification also provides numerical data for statistical analysis and has been used in previously in other studies.[15] Before grading biopsy specimens, two pathologists reached a consensus on the scoring of gastritis through interactive sessions using a multiheaded microscope. Subsequently all biopsy specimens were graded. According to this classification, the histopathological parameters were graded as follows.

The *H. Pylori* density was graded as follows:[14]

1) 0: none
2) 1: *H. Pylori* seen only in one place
3) 2: just a few *H. Pylori* seen
4) 3: dispersed *H. Pylori* seen in separate foci
5) 4: numerous *H. Pylori* in separate foci
6) 5: almost complete coverage of gastric surface by layer of *H. Pylori*
7) 6: uninterrupted coverage of gastric surface by a dense layer of *H. Pylori*

The degree of inflammatory activity was categorized

according to neutrophils density in gastric mucosa per biopsy:[14]

1) 0: no crypt involved
2) 1: one crypt is involved
3) 2: two crypts are involved
4) 3: up to 25% crypts are involved
5) 4: 25-50% crypts are involved
6) 5: > 50% crypts are involved
7) 6: all crypts are involved

Atrophy in gastric biopsies was graded as:[14]

1) 0: no change
2) 1: areas where a few gastric crypts are lost or changed into intestinal type metaplastic epithelium
3) 2: small areas in which gastric crypts are lost or changed into intestinal type metaplastic epithelium
4) 3: up to 25% gastric crypts lost or changed into intestinal type metaplastic epithelium
5) 4: 25-50% of gastric glands lost or changed into intestinal type metaplastic epithelium
6) 5: > 50% of gastric crypts lost or changed into intestinal type metaplastic epithelium
7) 6: only a few small areas in which gastric crypts are enduring

The extent of intestinal metaplasia was categorized according to the amount of gastric tissue replaced by metaplastic epithelium:[14]

1) 0: none
2) 1: only one crypt is replaced by metaplastic epithelium
3) 2: 1-4 crypts are replaced by metaplastic epithelium
4) 3: two dispersed foci
5) 4: many foci in one gastric biopsy
6) 5: >50% gastric epithelium is replaced by metaplastic epithelium
7) 6: only a small focal area of epithelium is not replaced by metaplastic epithelium

SPSS 17 was used to analyze the findings. Frequency and percentages represent quantitative variables. Relationship between density of *H. Pylori* and other variables was calculated through Spearman's rank correlation test. The findings were considered to be statistically significant if p value was found to be less than 0.05 ($p<0.05$).

Results

One hundred *H. Pylori* associated chronic gastritis patients were included in the study, out of which 68 were male and 32 cases were females. The median age of the patients was 54.15 years (range; 18-85 years). Figure 1 demonstrates the different grades of *H. Pylori* density observed in 100 biopsies of *H. Pylori* associated chronic gastritis.

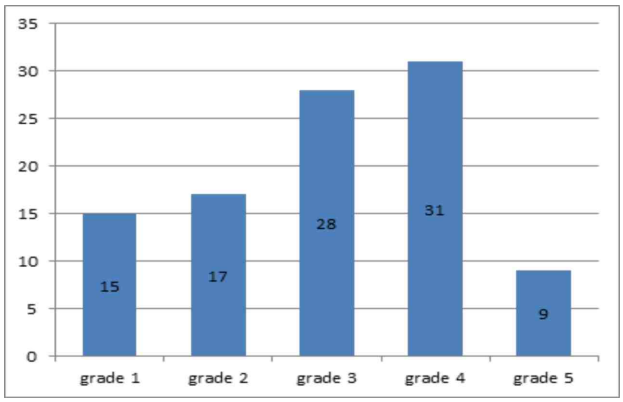

Fig 1: Grades of *H. Pylori* Density in Patients of Chronic gastritis (n=100)

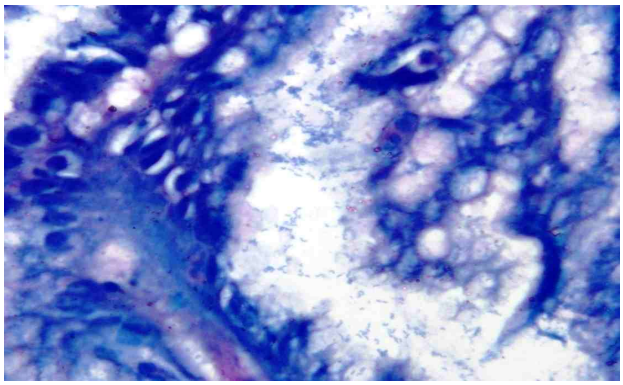

Fig 2: Photomicrograph of Gastric Biopsy Showing Nearly Complete Surface Covered by a Layer of *H. Pylori* (Grade 5 of *H. Pylori* Colonization) (Giemsa stain x 400)

Maximum number of 44 patients revealed no inflammatory activity (grade 0). Table I illustrates number of cases in each grade of *H. Pylori* density, showing different grades of neutrophilic activity.

Table I: Different Grades of Neutrophilic Inflammatory Activity and their Association with Grades of *H. Pylori* Density

Grades of *H. Pylori* density	Grades of inflammatory activity						Total (n)
	grade 0 (n)	grade 1 (n)	grade 2 (n)	grade 3 (n)	grade 4 (n)	grade 5 (n)	
Grade 1	8	2	2	2	0	1	15
Grade 2	13	2	1	1	0	0	17
Grade 3	13	2	3	8	1	1	28
Grade 4	10	0	7	10	4	0	31
Grade 5	0	1	0	4	4	0	9
Total (n)	44	7	13	25	9	2	100

Spearman's rank correlation revealed a significant weak positive ($r_{s=}$ 0.416) relation between grades of *H. Pylori* density and grades of neutrophilic inflammatory activity.

Among 100 patients, maximum number of 49 cases revealed no loss of gastric glands (grade 0 of atrophy). The detail of different grades of atrophy and their association with grades of *H. Pylori* density are evident in table II.

Table II: Grades of Atrophy and their Association with Grades of *H. Pylori* Density

Grades of *H. Pylori* density	Grades of atrophy						Total (n)
	grade 0 (n)	grade 1 (n)	grade 2 (n)	grade 3 (n)	grade 4 (n)	grade 5 (n)	
Grade 1	11	2	2	0	0	0	15
Grade 2	9	3	2	3	0	0	17
Grade 3	12	6	3	6	1	0	28
Grade 4	17	4	4	2	1	3	31
Grade 5	0	1	2	2	0	4	9
Total (n)	49	16	13	13	2	7	100

The statistical analysis done with Spearman's rank correlation suggested a weak positive relation ($r_{s=}$ 0.306) between grades of *H. Pylori* density and grades of atrophy.

Fifty one biopsies included in the study showed different grades of intestinal metaplasia and 49 biopsies revealed no intestinal metaplasia (grade 0). Detail of different grades is shown in table III.

Table III: Grades of Intestinal Metaplasia and their Association with Different Grades of *H. Pylori* Density

Grades of *H. Pylori* density	Grades of intestinal metaplasia						Total (n)
	grade 0 (n)	grade 1 (n)	grade 2 (n)	grade 3 (n)	grade 4 (n)	grade 5 (n)	
Grade 1	11	1	3	0	0	0	15
Grade 2	9	2	2	1	3	0	17
Grade 3	12	4	3	1	8	0	28
Grade 4	17	3	4	1	3	3	31
Grade 5	0	1	2	1	1	4	9
Total (n)	49	11	14	4	15	7	100

Spearman's rank correlation suggested a very weak positive correlation among grades of *H. Pylori* density and grades of intestinal metaplasia ($r_{s=}$0.287).

Discussion

H. Pylori organisms are the most important bacteria causing inflammation and chronic infection of the stomach. A number of studies show ambivalent results in relationship of *H. Pylori* density and degree of activity, atrophy and intestinal metaplasia. In this study we determined the density of *H. Pylori* semi quantitatively and found a weak positive correlation between bacterial density and these pathological findings of chronic gastritis.

In the present study, highest percentage of cases (n=59) was found in moderate grade *H. Pylori* density followed by mild and marked grades. These findings are similar to results of a study done at Mayo hospital.[16] Some authors suggested highest percentages in mild grade[17]while some in marked grade of *H. Pylori* density.[18] Number of factors contribute to these discrepancies including difference in *H. Pylori* strains, sample size and study design.

'Activity' is a variable component of *H. Pylori* linked chronic gastritis. Activity consists of numbers of neutrophils within the lamina propria, intraepithelial location and intraluminal location to form pit abscesses. In current study, the inflammatory activity was found to be 56% with maximum number of patients in moderate grade. In contrast 83% neutrophilic activity was reported in a study done in 2008 in Japan.[19] The present study shows a weak positive association between *H. Pylori* density and activity of chronic gastritis (r_s=0.416) and the results are similar to few other studies which also suggest that the neutrophilic activity shows a direct association with the density of *H. Pylori.*[19,20,21] The possible cause is that *H. Pylori* are an effective source of mediators that induce activation and chemotaxis of neutrophils. The variability of different strains of *H. Pylori* which induces upregulation of CD11b/CD18, chemotaxis of neutrophils along with oxidative burst response in neutrophils.[22] The extent of neutrophil activation and recruitment is determined by various factors like bacterial colonization, virulence, persistence, and the resultant innate and acquired host immune responses.[23]

It is well established fact that *H. Pylori* are involved in development of atrophy and intestinal metaplasia.[24] In our study, there was an increase in grades of the atrophy with the increasing grades of *H. Pylori* density in the gastric mucosa ($r_{s=}$ 0.306). The results are similar to other studies which also show a statistically significant and positive correlation between the intensity of *H pylori* and the degree of atrophy.[25,26,27]

Among the many risk factors, *H. Pylori* infection is considered as the most important risk factor of intestinal metaplasia.[28] The present study showed a statistically significant but very weak relation

between the density of *H. Pylori* and intestinal metaplasia (r_s=0.287). Two other studies also determined a significant correlation between density of *H. Pylori* and degree of intestinal metaplasia.[29,30] In contrast to the results of present study, another study documented statistically significant drop in the rate of *H. Pylori* colonization density with the increase in intestinal metaplasia.[31] The possible explanations are, because intestinal metaplasia is often focal process, *H. Pylori* may stay alive on the gastric epithelium in the surroundings of intestinal metaplasia. Moreover, there is a fact that the experience of the endoscopist affects the detection rate of intestinal metaplasia.[32]

The limitations of present study are that it is performed in a single institution based with limited number of cases, although the patients come from variety of ethnic groups and socioeconomic backgrounds. Large scale multicenter studies may be of help to improve the statistical power of this study.

Conclusion

After semi quantitative determination of histopathological parameters of *H. Pylori* associated chronic gastritis, it is concluded, that, the greater the density of *H. Pylori*, the larger the degrees of neutrophilic activity, intestinal metaplasia and atrophy. Comprehensive histopathological classification can be used in gastric biopsies to improve assessment and avoid minor degrees of alteration.

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