A HISTOPATHOLOGIC ANALYSIS OF CHRONIC INFLAMMATORY INFILTRATE IN PATIENTS OF H. PYLORI ASSOCIATED CHRONIC GASTRITIS

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ABSTRACT

Objective: To determine the relationship between H. pylori density with severity of chronic inflammatory infiltrate.

Study Design: A cross-sectional study.

Place and Duration of Study: The study was carried out in the Department of Pathology (Histopathology), Army Medical College, National University of Sciences and Technology (NUST) Islamabad, from Nov 2011 to Nov 2012.

Methodology: Gastric antral biopsies of H. pylori associated chronic gastritis were included in the study. Demographic characteristics and relevant clinical information were collected. First hundred biopsies of H. pylori associated chronic gastritis were assessed for density of H. pylori and chronic inflammatory infiltrate. Histopathological features like lymphoid aggregates, ulcer slough, superficial epithelial damage, dysplasia and nuclear reactive changes were simply assessed in case of their presence or absence.

Results: A significant moderate positive correlation was found between grades of H. pylori and chronic inflammatory infiltrate (rs= 0.636). Insignificant correlation was found with lymphoid aggregates, superficial epithelial damage, dysplasia and nuclear reactive changes.

Conclusion: In conclusion this study corroborated the determination of histopathological parameters and depicted that, the greater the density of H. pylori infection, the greater the degrees of chronic inflammatory infiltrate.

Keywords: Chronic gastritis, Chronic inflammatory infiltrate, H. Pylori.

INTRODUCTION

Gastritis is an inflammatory condition of the gastric mucosa. Gastritis can be classified based on the underlying etiology. H. pylori gastritis is a primary infection of the stomach and is the most common cause of chronic gastritis. It is estimated that half of the world’s population is infected with H. pylori. H. pylori gastritis occurs globally, but the prevalence varies greatly among countries and among population groups within the same country. In the developing countries like Pakistan, India, Bangladesh and Thailand, infection with H. pylori is more common among general population and is acquired at an early age. According to a study carried out in Pakistan 84 percent patients with chronic gastritis had H. pylori infection.

Histological changes of chronic gastritis include lymphocytes, plasma cells infiltration and other inflammatory cells in lamina propria. Neutrophilic infiltration is seen in active cases of disease. Lymphoid aggregate with germinal centre can also be observed. Chronic H. pylori gastritis exhibits more prominent gastritis in the antrum than in the corpus. The Sydney System is a novel classification and grading of gastritis that was devised by a group of experts at the 9th World Congress of Gastroenterology in Sydney, Australia in 1990. The Updated Sydney System has a scale of 0-3 for scoring the features of chronic gastritis. A detailed histopathological classification can be used, in order to improve assessment and avoid minor degrees of alteration. This classification also provides numerical data for statistical analysis.
H. pylori densities have been estimated previously according to subjective methods or using other techniques like PCR. We did not find any local literature regarding quantitative determination of H. pylori density and supporting the significant association of H. pylori load or density with severity of chronic inflammatory infiltrate. In order to address this issue the present study was designed.

**PATIENTS AND METHODS**

This cross-sectional study was conducted in the Department of Pathology, Army Medical College, National University of Sciences and Technology, Islamabad Pakistan between Nov 2011 and Nov 2012. One hundred gastric antral biopsies of H. pylori associated chronic gastritis patients of all ages and both sexes were included in the study. The biopsies were received from the Gastroenterology unit of Military Hospital, Rawalpindi. The patients included in the study were both serving and retired military persons as well as civilians living in Rawalpindi and its periphery. Gastric biopsies of patients who were receiving or had received H. pylori eradication treatment were excluded. Specimens were taken as a whole in 10% formal saline and were processed in Histopathology laboratory. Haematoxylin and Eosin (H&E) stain for routine histology and Giemsa stain was used for demonstration of H. pylori. The Updated Sydney System uses a scale of 0-3 for grading histopathological parameters. In order to improve assessment, a detailed classification resulting in a score from 0-6 is used in the present study (none, 0; mild, 1-2; moderate, 3-4; severe, 5-6). Under microscope following histopathological parameters were assessed and graded as follows:

**Density of H. pylori colonization:**
- 0: none
- 1: H. pylori found only in one place after a careful search
- 2: only a few H. pylori found
- 3: scattered H. pylori found in separate foci
- 4: numerous H. pylori in separate foci
- 5: nearly complete gastric surface covered by layer of H. pylori
- 6: continuous gastric surface covered by a thick layer of H. pylori

**Chronic Inflammatory Infiltrate:**
- 1: scattered chronic inflammatory cells<10 cells/ high power field
- 2: scattered chronic inflammatory cells>10 cells/ high power field
- 3: some foci with dense chronic inflammatory cells
- 4: diffuse infiltration with dense chronic inflammatory cells
- 5: nearly the whole mucosa contains dense infiltrate which separates gastric glands
- 6: entire mucosa contains a dense chronic inflammatory cell infiltrate.

**Non Quantitative Variables**
Non quantitative histopathological features like lymphoid aggregates, ulcer slough, superficial epithelial damage and nuclear reactive changes were not graded, but simply assessed in case of their presence or absence. Presence or absence of dysplasia was also noted.

**Statistical Analysis**
Data was analyzed using SPSS version 17. Descriptive statistics were used to describe the results. Spearman’s rank correlation was calculated to study relationship between H. pylori density and other variables. The experimental findings were considered statistically significant if p value was less than 0.05 (p<0.05).

**RESULTS**
One hundred patients of H. pylori associated chronic gastritis were included in the study, out of which 68% were male and 32% were females. The median age of the patients was 54.15 years (range: 18-85 years). Maximum number of cases (31%) was found to have grade 4 of H. pylori density. The next frequency (28%) was observed in grade 3. Overall 59% cases were found to have grade 3 and 4 of H. pylori density. Grade 1 and 2 of H. pylori density was found in 32% cases. Only
9% cases demonstrated marked H. pylori colonization.

The Table-1 shows the different grades of chronic inflammatory infiltrate among 100 patients of chronic gastritis and their association with grades of H. pylori density. Maximum number of 48% patients was observed to have grade 3 of chronic inflammatory infiltrate. Out of these 48%, maximum of 23% cases were found to be associated with grade 3 H. pylori density. Second highest frequency of 29% cases was found to have grade 4 of chronic inflammatory infiltrate. Seventeen out of these 29% cases were also associated with grade 4 of H. pylori density. Minimum number of 5% patients was observed to have grade 1 and 5 of chronic inflammatory infiltrate. There was a moderate and significant correlation between grades of H. pylori density and grades of chronic inflammatory infiltrate (rs = 0.636) (p = 0.001).

Photomicrograph of gastric biopsy demonstrating grade 4 H. pylori density and photomicrograph of a gastric biopsy indicating grade 4 chronic inflammatory infiltrate with formation of lymphoid aggregate in H. pylori associated chronic gastritis is shown in Fig-1.

Among the non quantitative variables 20% cases exhibited lymphoid aggregate formation with or without germinal centers. Out of these 20 cases, 9 patients were of grade 4 H. pylori density. There was insignificant correlation between H. pylori density and formation of lymphoid aggregates (rs = 0.105 p = 0.299).

Similarly insignificant correlation was noticed between H. pylori density grades and dysplasia (rs = 0.185 p = 0.066). Maximum patients (7 out of 15) showing dysplasia were observed in higher grades (grade 4) of H. pylori density. The details of other non quantitative variables are shown in Table-2.

**DISCUSSION**

Chronic gastritis is one of the most common chronic conditions of mankind. H. pylori organisms, highly prevalent pathogens, are the major causative bacteria leading to chronic infection of the stomach. In Pakistan the prevalence of H. pylori gastritis is as high as 87.03%. The mean age of H. pylori associated
gastritis was 54 years. There was no significant association between H. pylori density and age which is comparable to the findings of a study done in Karachi in which infection was frequent in forties and fifties and no significant association was found between age and H. pylori infection\textsuperscript{12}. The present study demonstrated that gender has some influence on H. pylori associated chronic gastritis. Females are less likely to be affected as compared to males. The results are comparable to a local study\textsuperscript{11}. The results of male predominance are also in accordance to a study in India in which male to female ratio was 3:1 whereas in this study the ratio was 2.2:1\textsuperscript{13}.

In this study we determined the density of H. pylori. Increased bacterial density of H. pylori is associated with a significant reduction in the eradication rate after H. pylori eradication treatment. This correlation provides additional support for the clinical importance of bacterial density as a factor in H. pylori eradication\textsuperscript{14}. The literature regarding frequency of different grades of H. pylori density is inconsistent. In the present study highest percentage of cases (n=59) was found in grade 3 and 4 of H. pylori density. These findings are similar to results of another study in which highest percentage i.e. 72.2\% was observed in moderate grade of H. pylori colonization followed by mild and marked grades\textsuperscript{1}. Some authors suggested highest percentages in mild grade\textsuperscript{15} while some in marked grade of H. pylori density\textsuperscript{16}. Number of factors contribute to these discrepancies including difference in H. pylori strains, sample size and study design.

Gastric inflammation is an invariable finding in patients infected with H. pylori. The presence of chronic inflammatory infiltrate comprising of T-lymphocytes and plasma cells in H. pylori chronic gastritis are due to antigen specific cellular and humoral immune mechanisms. In the majority cases of acute infection by H. pylori the host immune response fails to eliminate the infection and over the next 3 or 4 weeks there is a gradual accumulation of chronic inflammatory cells that come to dominate the histological picture. The possible cause is that H. pylori cause gastric epithelial damage and the factors released recruit monocytes and lymphocytes. These cells release pro-inflammatory mediators which act as antigen presenting cells to initiate specific immunity (T-lymphocytes and plasma cells)\textsuperscript{17}.

In the current study it was found that density of H. pylori in biopsy proven gastritis is positively correlated with chronic inflammatory infiltrate in the lamina propria (rs=0.636). Some previous data also support this finding. A study conducted by Siddiqui et al at Dow Diagnostic Reference and Research Laboratory, showed that...
the number of H. pylori in the antral mucosa is significantly associated with the quantity of mononuclear inflammatory cells. Comparable with these studies, two other studies suggested a statistically significant relationship between intensity of H. pylori and the degree of chronic inflammation.

In the present study all the cases exhibited different grades of chronic inflammatory infiltrate, with highest percentage of cases (n=77) noted to have grade 3 and 4 of chronic inflammation. The results are discordant with two other studies in which maximum patients were observed in severe and mild grades of chronic inflammation respectively. The possibility of the discrepancy of frequency may be due to different study design and number of cases. H. pylori are genetically heterogeneous and all strains may not play the same role in the development of inflammation. Strains containing a group of genes named Cag pathogenicity islands induce a greater degree of inflammation than strains lacking these genes.

Lymphoid follicles are a common feature of H. pylori associated gastritis. Lymphoid follicles may result from chronic antigenic stimulation and present a specific immune response directed against H. pylori. The association of H. pylori infection with lymphoid follicle formation and gastric mucosa-associated lymphoid tissue (MALT) lymphoma is well-known. In our study very weak association was noted between lymphoid follicle formation and H. pylori density.

The frequency of lymphoid follicle formation with or without germinal centre formation in our study was 20%. In the survey done in Iran lymphoid follicles were present in 50% of the H. pylori infected chronic gastritis patients, quite different from the report by Chen et al in 2002 which was 76%. These differences in frequencies between other previously published data and our study might result from the factors that the biopsy sites may have been different and the number of biopsy specimens taken varied in the different studies.

Our study revealed that 15 out of 100 cases had dysplasia. Although a very weak positive correlation was found between H. pylori density and dysplasia, maximum number of patients was associated with higher grades of H. pylori density. So it may be assumed that higher grades of H. pylori density may be associated with dysplasia if we increase the number of cases. As dysplasia is a pre malignant condition, therefore its recognition in the biopsy specimens is of great importance in giving warning of possibility of a co-existing carcinoma and indicating that the patient may be at higher risk for the development of gastric cancer.

CONCLUSION

In conclusion this study corroborated determination of histopathological parameters and depicted that, the greater the density of H. pylori, the greater the degrees of chronic inflammatory infiltrate in chronic gastritis.

Although large number of non invasive procedures for detection of H. pylori are available, gastric biopsy is still the gold standard, because this is the only way by which along with density of H. pylori one can adequately assess the degree of severity of histopathological parameters.

Intra gastric bacterial load can affect the success of H. pylori eradication therapy. Identifying patients with high bacterial loads before treatment and making adjustments of therapeutic regimens accordingly may further improve the efficacy of eradication therapy.

Conflict of Interest

This study has no conflict of interest to declare by any author.

REFERENCES
