ANTRAL GASTRIN CELL IN HELICOBACTER ASSOCIATED GASTRITIS : QUANTITATIVE IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

The number of antral gastrin cells (G cells) and their immunostaining pattern was evaluated in 28 gastroscopic biopsies without evidence of Helicobacter pylori organisms (HPOS) as a control group, and 42 gastroscopic biopsies with evidence of HPOs as a test group. There was no significant difference in number of G cells per gland in relation to the presence or absence of HPOs, degree of inflammation, type of gastritis and activity of inflammation (P>0.05). The intensity of immunostaining of G cells was stronger in association with HPOs positive group (++) than the negative group (+). The intensity of G cell staining was not affected by the severity of infection of HPOs. The intensity of immunostaining of G cells was higher in active than quiescent gastritis especially when associated with HPOs. The degree of gastritis has a direct effect on the intensity of immunostaining of G cells especially when associated with HPOs.

Keywords : gastritis, Helicobacter pylori, antral gastrin cells, immunohistochemistry.

INTRODUCTION

The role of Helicobacter pylori organisms (HPOs) in non ulcer dyspepsia, peptic ulcer disease and gastric neoplasia particularly mucosa associated lymphoid tissue (MALT) lymphoma is still not clear.[1-3] Many theories have been proposed but none has been universally accepted.[4-8] Traditionally the development of duodenal peptic ulcer has been associated with hyperacidity.[9] In a trial to

link the acid secretion with HPOs Levi et al. correlated the presence of HPOs with elevated serum gastrin levels.[10] At a latter date Sankey et al. tried to correlate the degree of HPOs associated gastritis with the number and intensity of antral gastrin cells (G cells) in the antral mucosa.[1]

The aim of this work is to document quantitatively the relation between the intensity of immunostaining pattern and the mean number of antral gastrin cells per gland with the amount of HPOs colonization and the degree and activity of inflammation in chronic gastritis.

MATERIAL AND METHODS

Seventy antral gastric biopsies were collected from the files of the Department of Pathology at King Abdulaziz University Hospital (KAUH). The paraffin blocks and the slides of these cases were retrieved. The slides have been stained by hematoxylin and eosin (H & E), Alcian blue-periodic acid Schiff reagent (AB-PAS) and Giemsa stain.

The slides were divided into two groups:-

1. Group (A) gastric biopsies negative for HPOs.
2. Group (B) gastric biopsies positive for HPOs.

The latter group was graded according to the amount of colonized organisms on the surface mucosa as few, moderate and plentiful using H & E and Giemsa stain.[11-12]

All the slides of both groups were assessed by a single observer for:

1. Type of gastritis (superficial or atrophic)
2. Grade of gastritis (1 = mild, 2 = moderate, 3 = severe).
3. The activity of chronic gastritis which was assessed by the presence of intraepithelial neutrophils (0 = quiescent, 1 = active).

For the immunostaining of antral gastrin cells, sections were cut from the paraffin blocks of both groups, mixed and stained in batches for gastrin according to Sankey et al., using rabbit antigastrin antibody with standard peroxidase-antiperoxidase method purchased from Shandon Scientific Ltd. Company (London). The number of G cells per gland was counted.

The selected glands were 200-300um in diameter and had complete regular transverse section. In few

cases the glands were small, 300um in diameter in which the number of G cells were counted using Leitz micrometer. The mean number of G cells was calculated and the significance was estimated using the studentt test and the two tailedt test. The intensity of staining was evaluated subjectively in relation to the control group and graded (+,++,+++). It was analyzed statistically using the X^2 test.

RESULTS

The number of antral gastric biopsies negative for HPOs was 28 and positive for HPOs was 42 antral gastric biopsies. The result was summarized in Table 1.

The mean number of G cells in the HPOs positive group (10±2.3) was numerically higher than that of the HPOs negative group (8.3±0.9), however statistically insignificant (P>0.05). Concerning the intensity of immunostaining of G cells in general, it was observed that the mean intensity was significantly greater (++) in biopsies positive for HPOs (Fig. 1 & 2) than the mean intensity (+) of the HPOs negative biopsies (Fig. 3). The staining pattern of G cells was in the form of light to dark brown diffuse cytoplasmic staining.

On considering the grade of gastritis, the mean number of G cells in the HPOs negative group did not vary significantly in the different grades of gastritis as it was almost the same (G1, 8.3±0.9) & G2, 8.75±0.2 & G3, 8±3.6). While in the HPOs positive groups there was a prominent increase in the mean number of G cells in the moderate degree of gastritis (11±2.3) but is was statistically insignificant (P>0.05). For the intensity of staining of G cells, it was increased in all grade of HPOs positive gastritis in comparison with the same grades in HPOs negative group.

There was no significant statistical difference in the number (P>0.05) and staining pattern of G cells (++) in relation to the degree of HPOs colonization of the mucosa (G1, 9±0.5 & G2, 10.5±2.1 & G3, 10.1±3.7). The detection of HPOs was more accurate and easier by Giemsa stain (Fig. 4) than by H & E stain (Fig. 5), particularly when the organisms were scanty. The use of Giemsa stain leads to improvement in the detection of HPOs by 8.5% which were diagnosed as HPOs negative by H & E stain (personal observation).

The activity of gastritis has no influence on the number of G cells in both HPOs positive group (active, 9.7±3.1 & quiescent, 9.3±1.8) (P>0.05) and HPOs negative group (active, 9.1±0.6 & quiescent, 8.1±1.6) (P>0.05). The intensity of immunostaining of G cells was significantly increased in the HPOs positive group (++) and HPOs negative group (+).

Also, the type of gastritis has no significant influence on the number of G cells (P>0.05) of both groups despite the little numerical difference between the superficial (HPOs negative 8.1±1.6 & HPOs positive 9.1±3.2) and deep (HPOs negative 8.3±2.5 & HPOs positive 9.7±3.2), however the intensity of staining was markedly increased in the deep gastritis (HPOs positive +++, HPOs negative ++) and generally more prominent in the HPOs positive group.

Table 1. The mean number of G cells with the standard deviation and intensity of immunostaining of antral gastrin cells.

	G cells mean number/gland	Staining intensity of G cells
HPOs:		
Negative	8.3±0.9	+
Positive	10±2.3	++
Grade of Gastritis:		
G1-HPOs negative	8.3±0.9	+
G1-HPOs positive	9.81±0.4	++
G2-HPOs negative	8.75±0.2	++
G2-HPOs positive	11±2.3	+++
G3-HPOs negative	8±3.6	++
G3-HPOs positive	9±3.75	+++
Amount of HPOs:		
1-Mild	9±0.5	++
2-Moderate	10.5±2.1	++
3-Plentiful	10.1±3.7	++
Activity of Gastritis:		
Q.HPOs negative	8.1±1.6	+
Q.HPOs positive	9.3±1.8	++
Ac.HPOs negative	9.2±0.6	+
Ac.HPOs positive	9.7±3.1	++
Level of Gastritis:		
Superficial (HPO-ve)	8.1±1.6	+
Superficial (HPO+ve)	9.1±3.2	++
Deep (HPO-ve)	8.3±2.5	++
Deep (HPO+ve)	9.7±3.1	+++

HPOs = Helicobacter pylori organisms
G cells = antral gastrin cells
Q = quiescent
Ac = active
HPO-ve = negative for Helicobacter pylori organisms
HPO+ve = positive for Helicobacter pylori organisms

Figure 1. Chronic active atrophic Helicobacter positive gastritis showing dark brown staining of G cells (+++) in actively inflamed gastric gland. (Immunostaining of Gastric x 100).

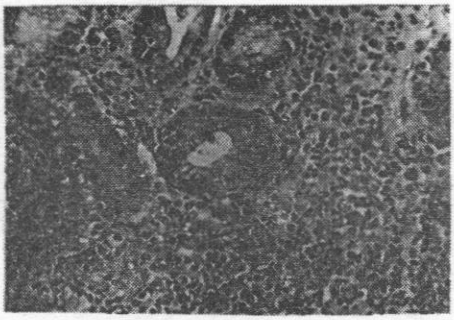

Figure 2. Gastric antral mucosa showing severe degree of inflammation and intense (+++) immunostaining of G cells from HPOs positive group. (Immunostaining for Gastris x 100).

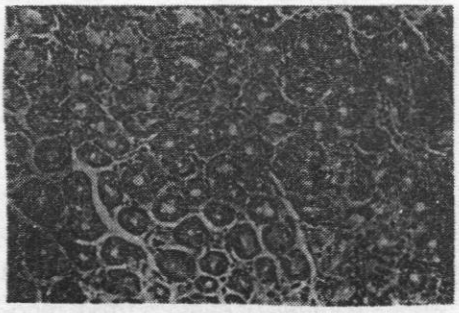

Figure 3. Chronic superficial Helicobacter negative gastritis showing pale brown (low) intensity staining of G cells (+). (Immunostaining for gastrin x 100).

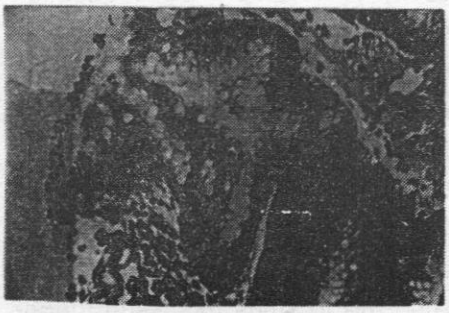

Figure 4. Marked surface colonization of the HPOs in the antral mucosa in a case of chronic active atrophic gastritis. (Giemsa stain x 100).

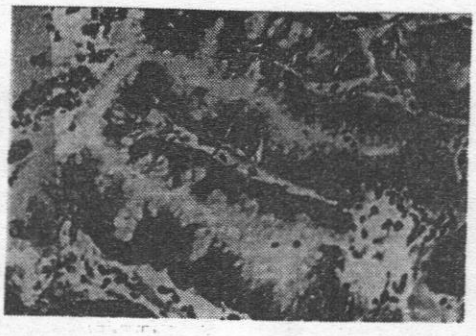

Figure 5. Markedly inflamed gastric antral mucosa showing moderate surface colonization of HPOs. (H & E stain x 100).

DISCUSSION

The controversy regarding the role of HPOs in non ulcer dyspepsia, peptic ulcer disease and gastric malignancy prompted me to investigate the possible mechanism of this role through the mediation of increased activity of G cells. This suggestion was initiated by the report of Levi et al who reported increased serum gastrin levels in the presence of HPOs.[10] This was further supported by Sankey et al. by reporting increased intensity of immunostaining of G cells in HPOs gastritis.1 This observation has stimulated me to study this issue further by comparing two groups of chronic gastritis with and without HPOs with regard to various parameters of gastritis as well as the amount of the HPOs organisms.

In general the present study showed an increase in the intensity of the immunostaining of G cells in all cases of chronic gastritis but the intensity was much more marked in the group of HPOs positive gastritis. On the contrary, there was no significant statistical difference in the number of G cells between the two groups. On further study of these results by using the various parameters of gastritis, it was found that though Marshall and Warren reported the association of HPOs only with moderate and severe gastritis, in the present work all grades of gastritis (mild, moderate, severe) were observed associated with HPOs which was in agreement with the reports of Dooley and Cohen, Price et al. and Wyatt and Dixon.[13-16]

The number of gastric cells in the HPOs negative group did not vary much in the different grades of inflammation as it was almost the same number in the different grades of gastritis denoting the lack of the effect on the degree of inflammation on the number of G cells. On the contrary, the HPOs group with the moderate degree of gastritis was associated with the highest number of these cells, which was more than that of the severe degree of inflammation. The same results were reported by Price et al., and Samket et al.[15-1]. The explanation of this difference was proposed by Bardy et al. who pointed out that though HPOs are superficial organisms and the severity of inflammation is more marked in the deeper zones of the mucosa where the G cells are mostly located, and therefore severe inflammation causes destruction of the G cells or disruption of their functions. Sincere the heavy colonization with HPOs if often associated with severe degree of inflammation, hence the paradoxical finding of reduction of G cells in relation to the degree of HPOs colonization on the gastric mucosa.

From the present study, it is clear that, there is no significant change in the number of G cells in different grades of inflammation in the control group (HPO-ve). While the number of G cells is increased in the HPO positive group, particularly in the moderate grade of gastritis, although statistically insignificant. These findings suggest that the Helicobacter pylori may have direct or indirect effect on the number of G cells other than the effect of inflammation itself. Although severe gastritis in the HPOs positive group was not associated with remarkable increase in the number of G cells, the intensity of staining was not much more increased in this degree of inflammation compared with the same degree in the HPOs negative group. Thus pointing to the possible effect of the severity of chronic inflammation as well as the HPOs on the activity of G cells and this could combine with gastric acidity to produce peptic ulceration.[1-18]

The increased intensity of staining of G cells with the severity of infection was further confirmed by Levi et al. and Smith et al. who reported raised serum gastrin and raised gastric acidity in the presence of HPOs thus indirectly supporting the possible interaction between HPOs and G cells.[10-19] Another explanation was provided by

Defize et al., who suggested that the HPOs bind to the parietal cells and inhibit them leading to reduced acidity and therefore hypergastrinemia.20 A more recent explanation was offered by Beardshall et al., who reported increased gastrin secretion as a result of decreased inhibition of gastrin secretion by the somatostatin which was found to be decreased in association with HPOs.[2]

It is to be concluded that the type of gastritis has a direct effect on the intensity of immunostaining of G cells but the additional presence of HPOs is related to a further, and significant, increase in the immunostaining. Also, the association of HPOs with superficial gastritis leads to the same results which support the relation between the active secretion of G cells and HPOs.

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