

Assessment of Cell Proliferation in Helicobacter Pylori Associated Gastric Epithelial Diseases

Neeraj Dhameja^{1*}, Ritambra Nada², D.K.Bhasin³, Kusum Joshi²

¹Dept. of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

²Dept of Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

³Dept of Gastroenterology, Post-Graduate Institute of Medical Education and Research, Chandigarh, India

Keywords: Atrophy, Carcinoma, Chronic Active Gastritis, Dysplasia, Intestinal Metaplasia, Helicobacter Pylori, Proliferative Index

ABSTRACT

Background: Alterations in cell proliferation and apoptosis as a result of Helicobacter Pylori infection can contribute to carcinogenesis. This study was planned to assess cellular proliferation during chronic active gastritis with or without H. Pylori, atrophy, intestinal metaplasia, dysplasia and carcinoma. Effect of H. Pylori eradication on cell proliferation was also studied.

Methods: Ki67 immunostaining was done to calculate proliferative index (PI) in H. pylori associated gastric diseases. Gastric biopsies of 160 patients with dyspepsia were selected comprising of 20 cases each of following groups 1) Normal control 2) H .pylori positive chronic active gastritis before and after treatment 3)H. pylori negative chronic active gastritis 4) Atrophy 5) Intestinal metaplasia 6) Dysplasia 7) Gastric adenocarcinoma

Result: There was increased proliferative index (PI) in H. pylori positive and negative chronic active gastritis as compared to normal controls. However, the PI in H. pylori positive chronic active gastritis was significantly higher than that of H. pylori negative chronic active gastritis. Increased proliferation was persistent in atrophy, intestinal metaplasia, dysplasia and carcinoma. There was significant increase in PI in foveolar regions in chronic active gastritis, intestinal metaplasia and dysplasia . After H. pylori eradication, there was marked reduction in PI.

Conclusion: H. pylori infection results in increased gastric epithelial cell proliferation which persists in the premalignant stages of atrophy, intestinal metaplasia, dysplasia and carcinoma. Presence of H. pylori augments proliferation resulting from inflammation and eradication of H. pylori reduces proliferation.. This increased proliferation may be one of the mechanism of H. pylori associated carcinogenesis.

***Corresponding author:**

Dr. Neeraj Dhameja, Assistant Professor, Dept of Pathology, Institute of Medical Sciences, Banaras Hindu University, Varanasi- 221005, India

Telephone: +91 - 7379645390

Email: doctorneeraj146@gmail.com,



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Introduction

Helicobacter pylori is a spiral shaped gram negative bacillus first described by Warren and Marshal in 1983 in gastric biopsies of patients with peptic ulcer and chronic active gastritis.^[1] It resides in and beneath the surface mucous layer of the gastric epithelium and secretes urease which protects it against acid by catalyzing urea hydrolysis to produce buffering ammonia. It is present worldwide but more frequent in developing countries and is associated with gastritis, peptic ulcer disease, gastric adenocarcinoma and lymphoid neoplasms like MALTOMA.^[2]

Long term *H.pylori* infection leads to multifocal atrophic gastritis characterized by loss of glandular tissue. Atrophy of the antral mucosa leads to the development of intestinal metaplasia (IM) which is linked to increased risk of gastric cancer.^[3-7] Hence, atrophy and IM are considered as premalignant lesions.^[8] Gastric carcinomas are classified as intestinal and diffuse types and *H.pylori* is related to both. Various bacterial and host factors have been described in the pathogenesis of *H.pylori* associated gastric epithelial diseases. The bacterial factors include tissue injury inducers like Cag A and Vac A (vacuolating cytotoxin). These factors act as proinflammatory substances and cause more intense gastric inflammation and greater cytokine production. Exact pathogenesis of gastric carcinoma is not known, however, it has been reported that *H.pylori* effects the cell kinetics with increased cell proliferation which may lead to development of carcinoma.^[9]

In the normal stomach, the proliferative compartment is located in the neck region of glands in the body, fundus and antrum. From this zone, the cells move towards the surface and deeper mucosal glands. Various studies have shown increased proliferation in *H.pylori* positive biopsies. Brenes et al^[10] analyzed *H.pylori* positive gastric biopsies and demonstrated that gastric mucosa infected with *H.pylori* is in a state of hyperproliferation which decreased after eradication of *H.pylori*. Bechi et al^[11] analyzed gastric corpus biopsies with *H.pylori* but no inflammation using tritiated thymidine and showed excessive replication suggesting direct effect of *H.pylori* on cell proliferation. Peek et al^[12] showed increased epithelial cell proliferation in *H.pylori* infection by immunohistochemical analysis of the proliferation associated antigen ki67. Cahill RJ et al^[13] identified an increased gastric epithelial cell proliferation associated with *H.pylori* infection which is reversed when the organism is eradicated. Murakami et al^[14] showed that gastric epithelial cell proliferation in the antrum and corpus is increased in *H.pylori* associated gastritis and eradication of the bacteria leads to the reversal of cell proliferation to normal. Cell proliferation is increased when atrophy is present and gastric dysplasia develops

against a background of atrophic gastritis and intestinal metaplasia. Increasing severity of dysplasia is associated with an expanding proliferating zone. Similarly Fan et al^[15], Tseng et al^[16], Ren et al^[17] and Peek RM et al^[18] found increased gastric epithelial cell proliferation in *H.pylori* infected gastritis. This increased cell proliferation is also present in the premalignant conditions like atrophy and IM. In the gastritis stage, the proliferation is reversible by eradication of *H.pylori*^[19], however, in the stages of atrophy and intestinal metaplasia, it cannot be reverted back to normal levels.

Since *H.pylori* has high prevalence in India, in this study, we evaluated cell proliferation in different gastric epithelial diseases associated with *H.pylori* by immunostaining with ki67.

Materials and Methods

All adult patients presenting with dyspeptic symptoms were enrolled for this study. After complete clinical evaluation, upper GI Endoscopy (Olympus GEX) was performed after explaining the procedure to the patient and obtaining a written consent. Detailed medical history was taken and patients on NSAIDs, steroids were excluded. The patients who were diagnosed to have chronic active gastritis due to *H. pylori* were treated using triple drug regimen and a repeat biopsy was taken after six weeks of therapy. Endoscopic antral biopsies were oriented on pieces of filter paper, fixed in 10% buffered formalin and sent to the Histopathology department. The biopsies were processed using standard processing protocol and multiple serials were stained with Haematoxylin & eosin.

Biopsies were evaluated by a group of three pathologists independently. Each biopsy was assessed for activity, chronic inflammation, atrophy, intestinal metaplasia, dysplasia and *H. pylori* according to the updated Sydney system.^[20] Biopsies with proper orientation were selected for further evaluation. The biopsies were grouped into eight groups of twenty each as follows

- 1) Normal control (NC, as per updated Sydney system)
- 2) Chronic active gastritis (CAG) without *H. pylori*
- 3) Chronic active gastritis with *H.pylori* (Pretreatment biopsies)
- 4) Chronic active gastritis with *H. pylori* (Post treatment biopsies)
- 5) *H.pylori* gastritis with atrophy
- 6) *H.pylori* gastritis with intestinal metaplasia
- 7) *H. pylori* gastritis with dysplasia
- 8) Gastric adenocarcinoma of the intestinal type

Proliferation was assessed by immunoperoxidase staining for ki67, a proliferation associated antigen using the MIB-

1 monoclonal antibody (Novacastra, dilution 1:100). At least 1000 cells in the three consecutive gastric foveolar epithelia, neck and glandular regions were counted in each case for assessing topographical distribution of proliferative cells. Cells showing distinctive brown staining of the nuclei were counted as positive. The mean number of positive cells per total of 1000 epithelial cells for each region and combination of these regions (foveolae F, neck N and glands G) was expressed as the Proliferative Index (PI).

Proliferative Index (PI) = $\text{ki67 positive cells} \times 100 / \text{Total cells (1000)}$

Statistical Analysis: Students unpaired t test was used for comparison of normal control and other groups (CAG with *H. pylori*, CAG without *H. pylori*, atrophy, intestinal metaplasia, dysplasia and carcinoma). Students paired t test was used for comparison of chronic active gastritis with *H. pylori* (pretreatment biopsies) and antral biopsies after *H. pylori* eradication therapy. P value less than 0.05 was considered significant.

Results

There were 120 males and 40 females in the age range of 20 to 75 years (mean 45.65 yrs). Endoscopically, duodenal ulcers were present in 83, gastric ulcers in 16 and 133 patients had antral gastritis. Biopsy Rapid urease test of patients with *H. pylori* was positive.

The proliferative indices (PI) of different groups were calculated topographically for foveolar, neck and gland regions and combination of these regions.

The cumulative PI of control group was 0.6% to 7.5% (mean 3.9%) (Fig. 1). The cumulative PI of chronic active gastritis without *H. pylori* was 1.03% to 22.3% (mean 7.57%) (Fig. 2) and with *H. pylori* was 9.06% to 29.54% (mean 16.68%) (Fig. 3) which was significantly higher ($p < 0.02$) and ($p < 0.00000$) respectively than the cumulative PI of control group. However, the cumulative PI of chronic active gastritis with *H. pylori* was significantly more than the cumulative PI of chronic active gastritis without *H. pylori* ($p < 0.00002$). The cumulative PI of chronic active gastritis with *H. pylori* reduced significantly (1.04% to 17%, mean 7.10%, $p < 0.00000$) (Fig. 4) after eradication therapy when compared with pretreatment PI. However, post treatment PI was still higher than that of normal control.

The cumulative PI of premalignant lesions i.e. atrophy (2.56% to 17.56%, mean 8.08%) (Fig. 5), intestinal metaplasia (7.1% to 34.23%, mean 15.7%) (Fig. 6), dysplasia (9.53% to 45.56%, mean 25.2%) (Fig. 7) and adenocarcinoma (33.18% to 71%, mean 38.60%) (Fig. 8) was significantly higher than that of normal controls and *H. pylori* negative CAG.

The PI was also calculated topographically for foveolae, neck and gland region. It was found that in *H. pylori* positive CAG, the PI in the foveolar region (1% to 6.6%, mean 3.29%) was significantly higher than that of normal control (0.1% to 3.7%, mean 0.92%, $p < 0.00000$) and *H. pylori* negative chronic active gastritis (0.4% to 4%, mean 1.62%, $p < 0.001$). This increased PI in the foveolar region was persistent and further increased in the stages of intestinal metaplasia (0.2% to 22.2%, mean 4.7%) and dysplasia (0.66% to 56.6%, mean 22.16%). (Table.1, Fig.9).

Discussion

Gastric carcinoma has been associated with *H. pylori* based on epidemiological and clinical investigation. Correa^[21] has suggested that *H. pylori* associated gastric carcinoma develops through a multistep process from chronic active gastritis (CAG) to atrophy, intestinal metaplasia, dysplasia and carcinoma. *H. pylori* infected gastric mucosa of patients with CAG is characterized by the infiltration of neutrophils and lymphocytes and is associated with varying degrees of atrophy and intestinal metaplasia in long standing infection which are premalignant lesions. Exact pathogenesis of *H. pylori* associated gastric carcinoma is not known, however, it has been hypothesized that alteration of cellular turnover may be one of the mechanism of *H. pylori* associated carcinogenesis. The present study was therefore designed to quantitate the cellular proliferation of gastric epithelial cells in various topographic regions of gastric mucosa through the spectrum of gastric epithelial lesions associated with *H. pylori* infection.

Cellular turnover in a living body consists of a balance between apoptosis and cell proliferation. Imbalance with increased proliferation and decreased apoptosis disrupts homeostasis and may be an early event in malignant transformation. Various studies have shown that *H. pylori* is associated with increased cell proliferation which reduces to normal after eradication of *H. pylori*.^[10,13,22,23] The increased proliferation is itself not sufficient for carcinoma, but the proliferating cells are susceptible to mutations which may lead to carcinoma. In this study, we used ki67 immunostaining to identify cells in the proliferative phase of the cell cycle. The monoclonal antibody ki67 reacts with human nuclear antigen (expressed only in cycling cells, not in quiescent cells) and the ki67 labelling index corresponds to the growth fraction of a cell population. Our data shows that there is a significant increase in proliferative index (PI) in biopsies with *H. pylori* positive CAG as compared to normal biopsies. On topographical analysis, it was observed that there was expansion of proliferative zone with increased PI in the foveolar and neck regions. Similar results were shown by Lynch et al^[22] and Panella et al^[24].

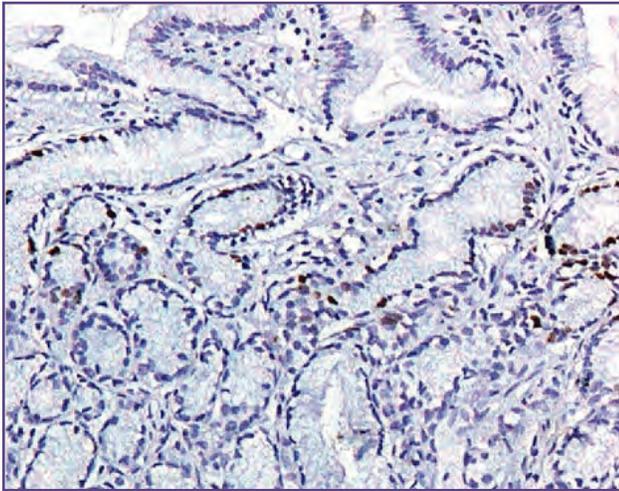


Fig. 1: Ki67 immunostaining in normal gastric antral biopsies

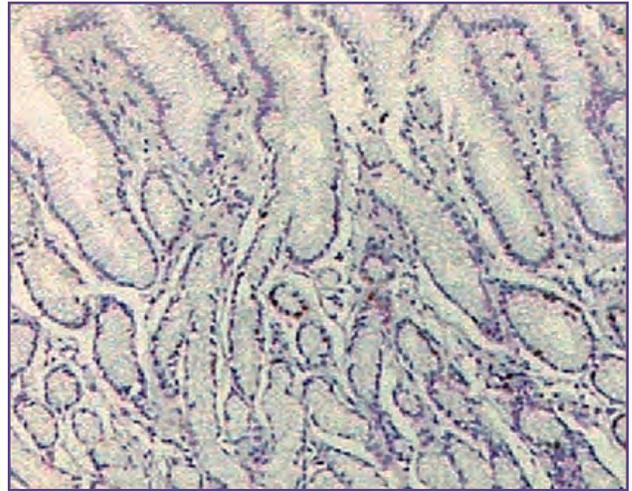


Fig.4. Ki67 immunostaining in posttreatment biopsies

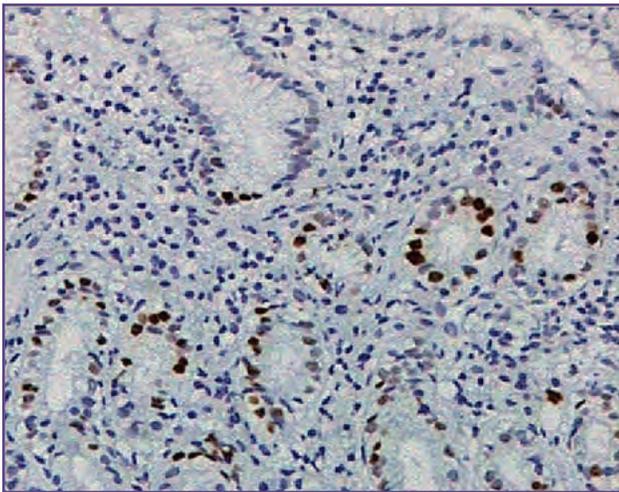


Fig.2: Ki67 immunostaining in H pylori negative CAG

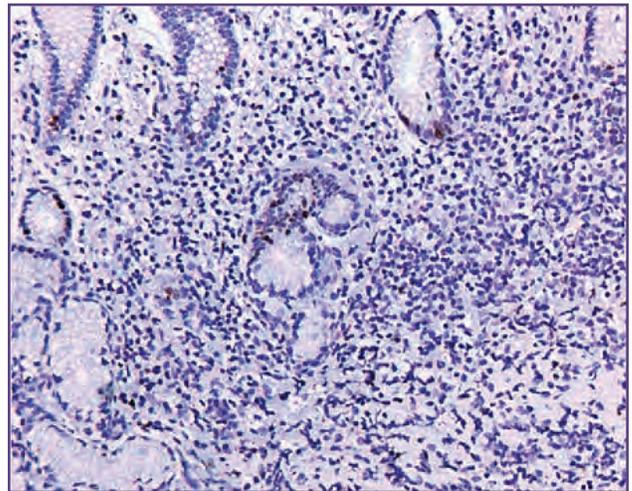


Fig.5: Ki67 immunostaining in antral biopsy with atrophy

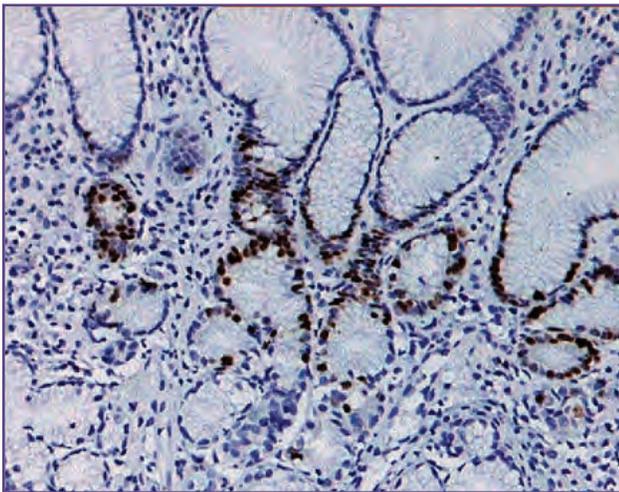


Fig.3: Ki67 immunostaining in H pylori positive CAG

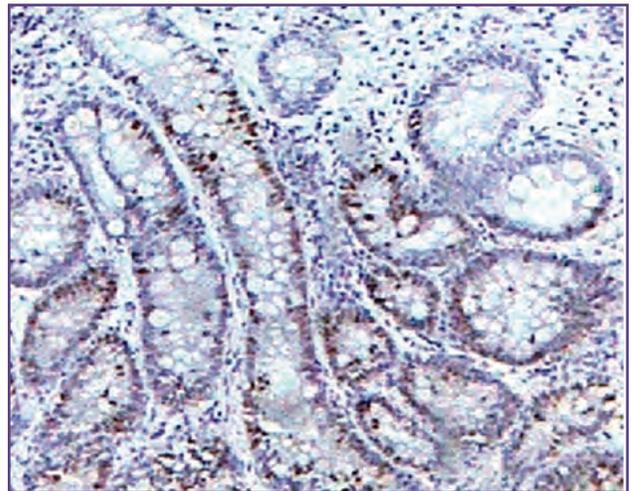


Fig.6: Ki67 immunostaining in antral biopsy with intestinal metaplasia

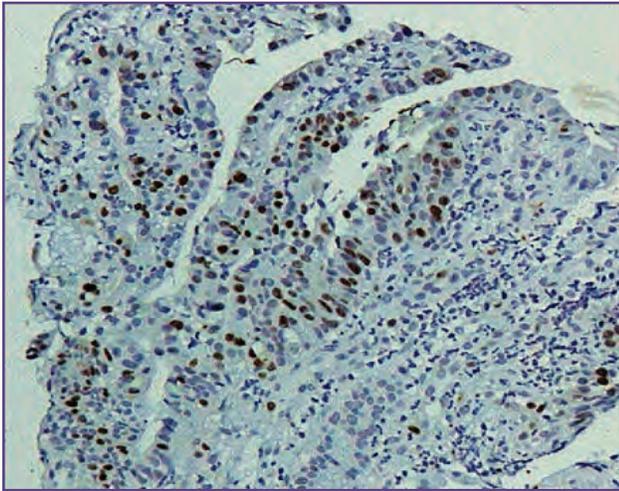


Fig.7: Ki67 immunostaining in antral biopsy with dysplasia

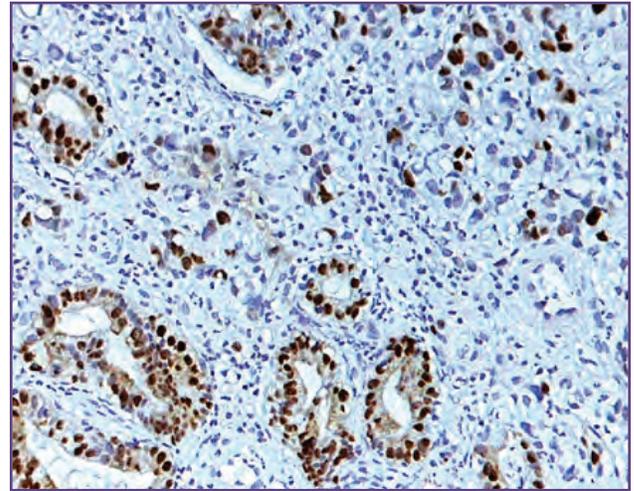


Fig.8: Ki67 immunostaining in moderately differentiated adenocarcinoma

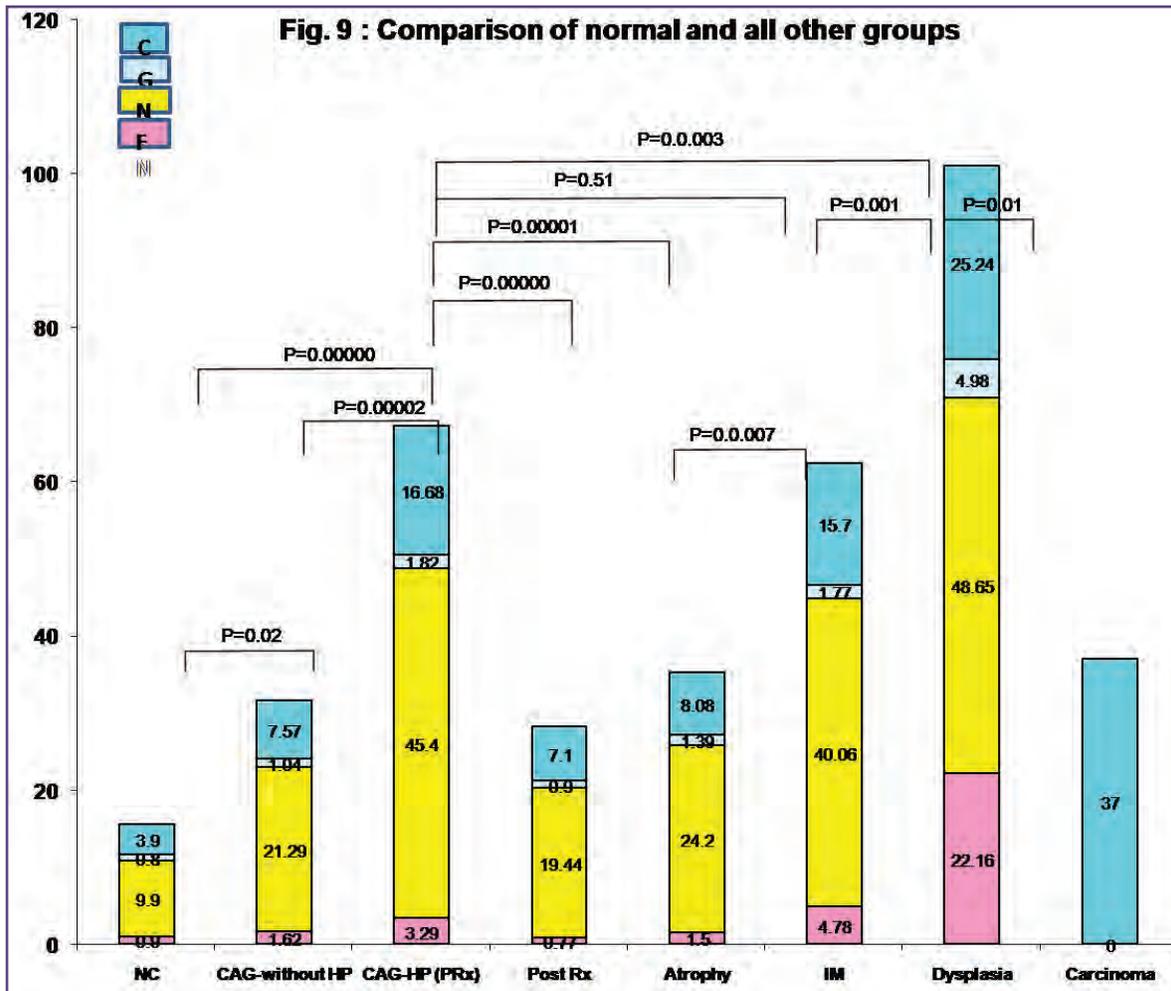


Fig. 9: Comparison of normal and all other groups

Table 1: Proliferative Indices (mean, topographical and cumulative) of Ki67 immunostained antral biopsies of all the groups.

| S.No | Groups | Foveolae | Neck | Gland | Cumulative |
|------|-----------------------------------|---------------|---------------|-------------|---------------|
| 1 | Normal control | 0.92 ± 0.9 | 9.97 ± 5.35 | 0.83 ± 1.10 | 3.90 ± 2.20 |
| 2 | CAG without H pylori | 1.62 ± 1.19 | 21.29 ± 15.50 | 1.04 ± 0.78 | 7.57 ± 6.06 |
| 3 | CAG with H pylori (Pre Treatment) | 3.29 ± 1.87 | 45.4 ± 14.62 | 1.82 ± 1.10 | 16.68 ± 5.98 |
| 4 | Post Treatment | 0.77 ± 1.26 | 19.44 ± 10.81 | 0.9 ± 1.73 | 7.10 ± 4.09 |
| 5 | H pylori Gastritis with atrophy | 1.50 ± 2.09 | 24.2 ± 11.75 | 1.39 ± 1.68 | 8.08 ± 4.9 |
| 6 | H pylori Gastritis with IM | 4.7 ± 4.99 | 40.06 ± 17.91 | 1.77 ± 3.16 | 15.70 ± 7.54 |
| 7 | H pylori Gastritis with Dysplasia | 22.16 ± 16.45 | 48.65 ± 16.63 | 4.98 ± 9.62 | 25.24 ± 10.47 |
| 8 | Gastric Adenocarcinoma | | | | 38.60 ± 16.65 |

Our data shows significant increase in PI in CAG without *H.pylori* with a similar expansion of proliferation compartment as compared to normal controls. However, there was significant difference in PI between *H.pylori* positive CAG and *H.pylori* negative CAG. These observations suggest that inflammation increases proliferation both in *H.pylori* positive and *H.pylori* negative CAG as compared to normal control, but *H.pylori* infection has a role in further augmentation of the proliferative response.

Bechi et al^[11] using ³H tritiated thymidine labelling found higher labelling index in *H.pylori* positive CAG biopsies compared to *H.pylori* negative CAG biopsies, however, the difference was not statistically significant. The result of the present study as well as the study by Lynch et al^[22] are contrary to the above. This difference may be due to the different methods used to evaluate cell proliferation. ³H thymidine labelling quantitates only the S phase fraction whereas ki67 quantitates growth fraction (all cycling cells in a population). It is apparent that increased cell turnover with a proportionate shortening of all phases of the cell cycle will not be reflected by a higher S phase fraction. Hence ki67 labelling may be considered a better proliferation marker than ³H thymidine labelling. Therefore, our findings along with those of Lynch et al^[22] support an association of *H.pylori* infection with both increased epithelial cell proliferative activity and expansion of the mucosal proliferative compartment.

In the present study, PI in antral biopsies showing atrophy was also quantitated. PI (combined and neck) was higher in areas of atrophy as compared to normal biopsies. The PI of foveolae and gland region was also higher in atrophic areas when compared to the normal but the difference was not statistically significant. However, the PI of atrophic region was statistically less than that of *H.pylori*

positive CAG. These observations suggest that chronic inflammation in atrophic areas is responsible for persistent increased proliferation.

In the present study, PI in areas of IM was found to be higher in all topographical areas (foveolae, neck and gland) of mucosa as compared to normal controls and was comparable to that of *H.pylori* positive CAG. Similar results were shown by Tseng et al^[16]. Ierardi et al^[25] demonstrated that hyperproliferation associated with IM is not reversed by *H.pylori* eradication, suggesting that proliferation of the metaplastic cells is no longer dependent on bacterial factors.

We also studied antral biopsies showing dysplasia and the PI (combined and topographical) was significantly higher in these dysplastic regions when compared with normal control and *H.pylori* positive CAG. However, PI was statistically increased in the foveolar region only when compared with *H.pylori* positive CAG and areas of IM respectively. This suggests that as there is progression from IM to dysplasia, the proliferative compartment shifts to the foveolar region (superficialisation of the proliferative compartment). We also studied PI in gastric adenocarcinomas of intestinal type and found higher PI in the carcinomatous areas as compared to NC and other groups. The combined PI in carcinoma was significantly higher than *H.pylori* positive CAG, atrophy, IM and dysplasia.

Our data to assess the response of proliferation to *H.pylori* eradication therapy performed on post treatment biopsies showed significant decrease in PI as compared to pretreatment biopsies. However, proliferation in post treatment biopsies was as high as *H.pylori* negative CAG, thereby suggesting a lowering of proliferation after eradication of *H.pylori*. The continuing chronic

inflammation that persists in post treatment biopsies could be responsible for the PI higher than normal controls. Lynch et al^[22] and Brenes et al^[10] have shown similar results.

Conclusion

In summary, *H.pylori* associated gastric carcinoma is a multistep process and evolves through the stages of chronic active gastritis to atrophy, intestinal metaplasia and dysplasia. The present study has shown that *H.pylori* infection is associated with increased proliferation in all regions of gastric mucosa as compared to normal controls. As there is progression from CAG to IM, dysplasia and carcinoma, cell proliferation increases progressively. Eradication of *H.pylori* markedly reduces cell proliferation but does not bring it down to normal levels, suggesting also a role of inflammation in proliferation.

Funding

None

Competing Interests

None

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Since *H.pylori* has high prevalence in India, in this study, we evaluated cell proliferation in different gastric epithelial diseases associated with *H.pylori* by immunostaining with ki67.

Materials and Methods

All adult patients presenting with dyspeptic symptoms were enrolled for this study. After complete clinical evaluation, upper GI Endoscopy (Olympus GEX) was performed after explaining the procedure to the patient and obtaining a written consent. Detailed medical history was taken and patients on NSAIDS, steroids were excluded. The patients who were diagnosed to have chronic active gastritis due to *H.pylori* were treated using triple drug regimen and a repeat biopsy was taken after six weeks of therapy. Endoscopic antral biopsies were oriented on pieces of filter paper, fixed in 10% buffered formalin and sent to the Histopathology department. The biopsies were processed using standard processing protocol and multiple serials were stained with Haematoxylin & eosin.

Biopsies were evaluated by a group of three pathologists independently. Each biopsy was assessed for activity, chronic inflammation, atrophy, intestinal metaplasia, dysplasia and *H.pylori* according to the updated Sydney system.^[20] Biopsies with proper orientation were selected for further evaluation. The biopsies were grouped into eight groups of twenty each as follows

- 1) Normal control (NC, as per updated Sydney system)
- 2) Chronic active gastritis (CAG) without *H.pylori*
- 3) Chronic active gastritis with *H.pylori* (Pretreatment biopsies)
- 4) Chronic active gastritis with *H.pylori* (Post treatment biopsies)
- 5) *H.pylori* gastritis with atrophy
- 6) *H.pylori* gastritis with intestinal metaplasia
- 7) *H.pylori* gastritis with dysplasia
- 8) Gastric adenocarcinoma of the intestinal type

Proliferation was assessed by immunoperoxidase staining for ki67, a proliferation associated antigen using the MIB-

1 monoclonal antibody (Novacastra, dilution 1:100). At least 1000 cells in the three consecutive gastric foveolar epithelia, neck and glandular regions were counted in each case for assessing topographical distribution of proliferative cells. Cells showing distinctive brown staining of the nuclei were counted as positive. The mean number of positive cells per total of 1000 epithelial cells for each region and combination of these regions (foveolae F, neck N and glands G) was expressed as the Proliferative Index (PI).

Proliferative Index (PI) = ki67 positive cells x100/Total cells (1000)

Statistical Analysis: Students unpaired t test was used for comparison of normal control and other groups (CAG with *H. pylori*, CAG without *H. pylori*, atrophy, intestinal metaplasia, dysplasia and carcinoma). Students paired t test was used for comparison of chronic active gastritis with *H. pylori* (pretreatment biopsies) and antral biopsies after *H. pylori* eradication therapy. P value less than 0.05 was considered significant.

Results

There were 120 males and 40 females in the age range of 20 to 75 years (mean 45.65 yrs). Endoscopically, duodenal ulcers were present in 83, gastric ulcers in 16 and 133 patients had antral gastritis. Biopsy Rapid urease test of patients with *H. pylori* was positive.

The proliferative indices (PI) of different groups were calculated topographically for foveolar, neck and gland regions and combination of these regions.

The cumulative PI of control group was 0.6% to 7.5% (mean 3.9%) (Fig. 1). The cumulative PI of chronic active gastritis without *H. pylori* was 1.03% to 22.3% (mean 7.57%) (Fig. 2) and with *H. pylori* was 9.06% to 29.54% (mean 16.68%) (Fig. 3) which was significantly higher ($p < 0.02$) and ($p < 0.00000$) respectively than the cumulative PI of control group. However, the cumulative PI of chronic active gastritis with *H. pylori* was significantly more than the cumulative PI of chronic active gastritis without *H. pylori* ($p < 0.00002$). The cumulative PI of chronic active gastritis with *H. pylori* reduced significantly (1.04% to 17%, mean 7.10%, $p < 0.00000$) (Fig. 4) after eradication therapy when compared with pretreatment PI. However, post treatment PI was still higher than that of normal control.

The cumulative PI of premalignant lesions i.e. atrophy (2.56% to 17.56%, mean 8.08%) (Fig. 5), intestinal metaplasia (7.1% to 34.23%, mean 15.7%) (Fig. 6), dysplasia (9.53% to 45.56%, mean 25.2%) (Fig. 7) and adenocarcinoma (33.18% to 71%, mean 38.60%) (Fig. 8) was significantly higher than that of normal controls and *H. pylori* negative CAG.

The PI was also calculated topographically for foveolae, neck and gland region. It was found that in *H. pylori* positive CAG, the PI in the foveolar region (1% to 6.6%, mean 3.29%) was significantly higher than that of normal control (0.1% to 3.7%, mean 0.92%, $p < 0.00000$) and *H. pylori* negative chronic active gastritis (0.4% to 4%, mean 1.62%, $p < 0.001$). This increased PI in the foveolar region was persistent and further increased in the stages of intestinal metaplasia (0.2% to 22.2%, mean 4.7%) and dysplasia (0.66% to 56.6%, mean 22.16%). (Table.1, Fig.9).

Discussion

Gastric carcinoma has been associated with *H. pylori* based on epidemiological and clinical investigation. Correa^[21] has suggested that *H. pylori* associated gastric carcinoma develops through a multistep process from chronic active gastritis (CAG) to atrophy, intestinal metaplasia, dysplasia and carcinoma. *H. pylori* infected gastric mucosa of patients with CAG is characterized by the infiltration of neutrophils and lymphocytes and is associated with varying degrees of atrophy and intestinal metaplasia in long standing infection which are premalignant lesions. Exact pathogenesis of *H. pylori* associated gastric carcinoma is not known, however, it has been hypothesized that alteration of cellular turnover may be one of the mechanisms of *H. pylori* associated carcinogenesis. The present study was therefore designed to quantitate the cellular proliferation of gastric epithelial cells in various topographic regions of gastric mucosa through the spectrum of gastric epithelial lesions associated with *H. pylori* infection.

Cellular turnover in a living body consists of a balance between apoptosis and cell proliferation. Imbalance with increased proliferation and decreased apoptosis disrupts homeostasis and may be an early event in malignant transformation. Various studies have shown that *H. pylori* is associated with increased cell proliferation which reduces to normal after eradication of *H. pylori*.^[10,13,22,23] The increased proliferation is itself not sufficient for carcinoma, but the proliferating cells are susceptible to mutations which may lead to carcinoma. In this study, we used ki67 immunostaining to identify cells in the proliferative phase of the cell cycle. The monoclonal antibody ki67 reacts with human nuclear antigen (expressed only in cycling cells, not in quiescent cells) and the ki67 labelling index corresponds to the growth fraction of a cell population. Our data shows that there is a significant increase in proliferative index (PI) in biopsies with *H. pylori* positive CAG as compared to normal biopsies. On topographical analysis, it was observed that there was expansion of proliferative zone with increased PI in the foveolar and neck regions. Similar results were shown by Lynch et al^[22] and Panella et al^[24].

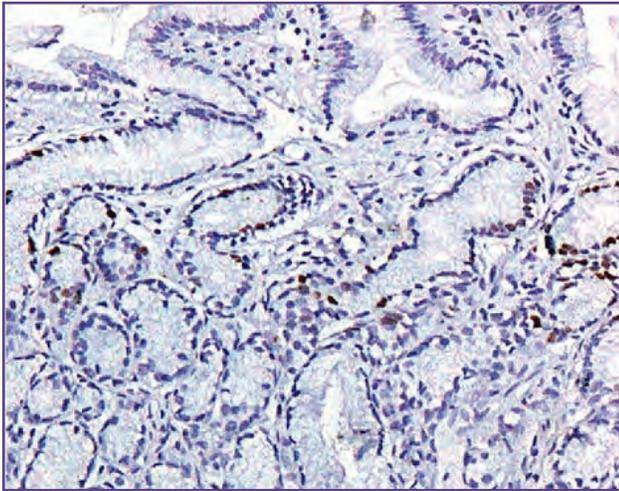


Fig. 1: Ki67 immunostaining in normal gastric antral biopsies

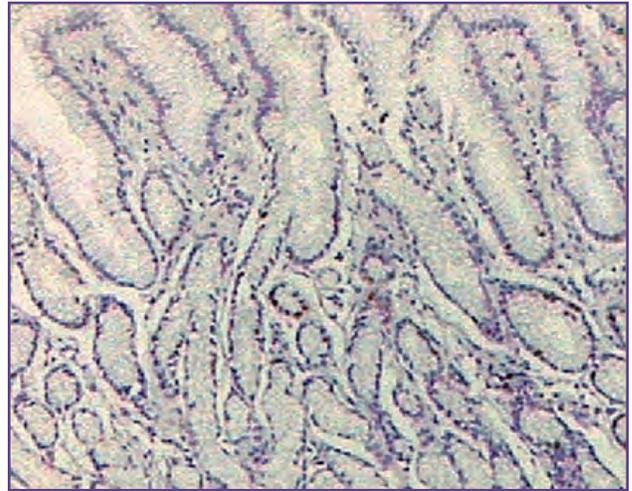


Fig.4. Ki67 immunostaining in posttreatment biopsies

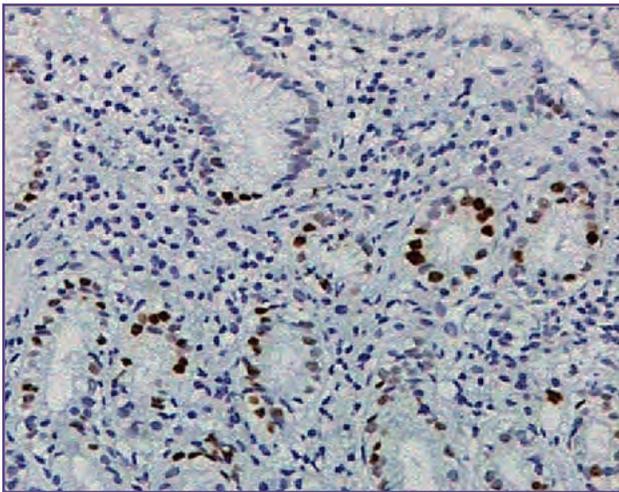


Fig.2: Ki67 immunostaining in H pylori negative CAG

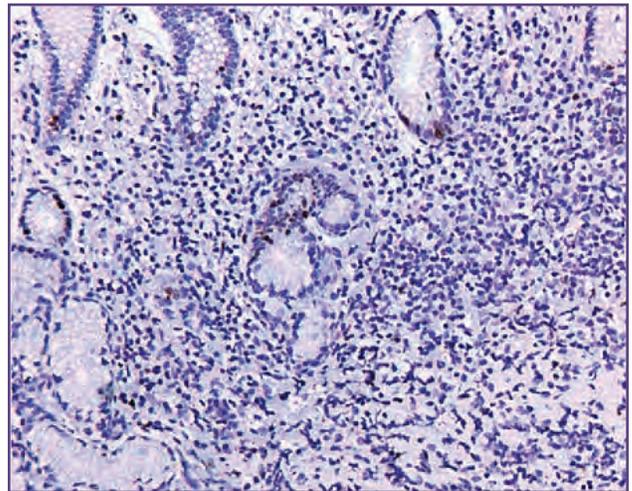


Fig.5: Ki67 immunostaining in antral biopsy with atrophy

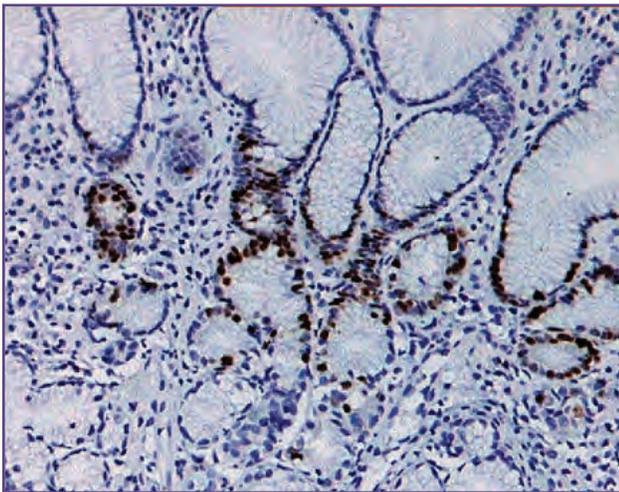


Fig.3: Ki67 immunostaining in H pylori positive CAG

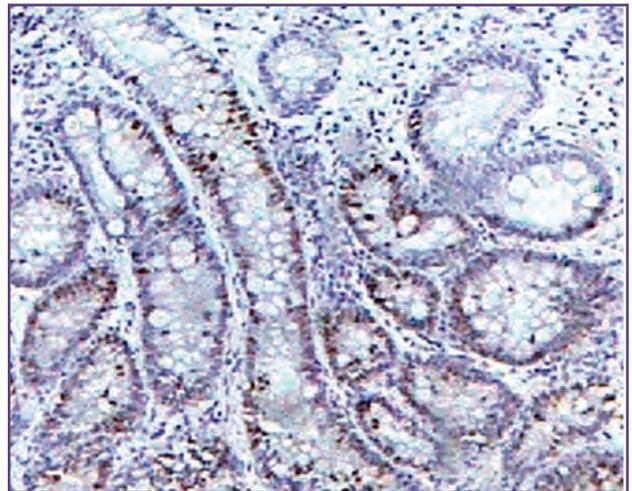


Fig.6: Ki67 immunostaining in antral biopsy with intestinal metaplasia

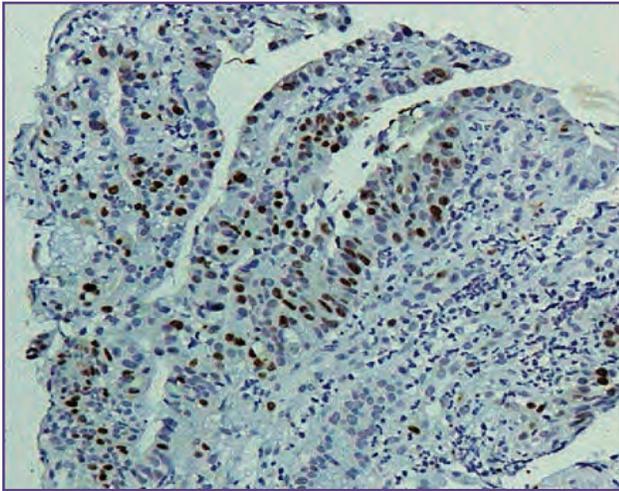


Fig.7: Ki67 immunostaining in antral biopsy with dysplasia

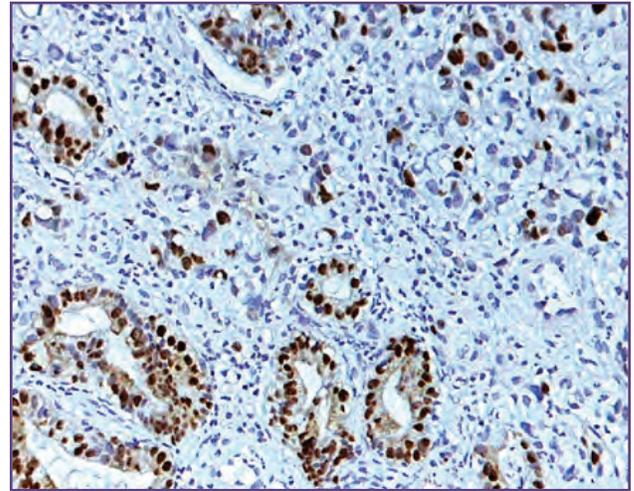


Fig.8: Ki67 immunostaining in moderately differentiated adenocarcinoma

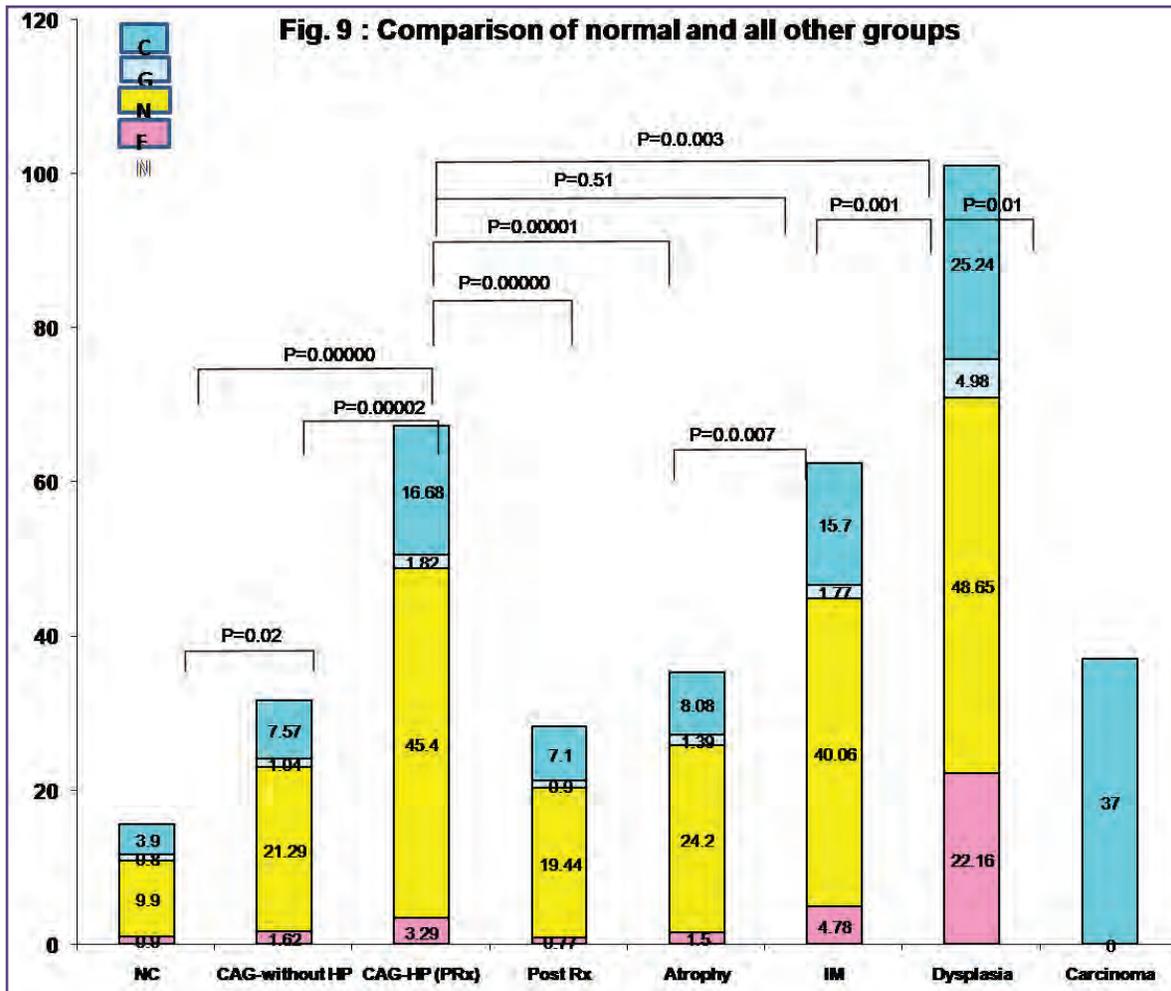


Fig. 9: Comparison of normal and all other groups

Table 1: Proliferative Indices (mean, topographical and cumulative) of Ki67 immunostained antral biopsies of all the groups.

| S.No | Groups | Foveolae | Neck | Gland | Cumulative |
|------|-----------------------------------|---------------|---------------|-------------|---------------|
| 1 | Normal control | 0.92 ± 0.9 | 9.97 ± 5.35 | 0.83 ± 1.10 | 3.90 ± 2.20 |
| 2 | CAG without H pylori | 1.62 ± 1.19 | 21.29 ± 15.50 | 1.04 ± 0.78 | 7.57 ± 6.06 |
| 3 | CAG with H pylori (Pre Treatment) | 3.29 ± 1.87 | 45.4 ± 14.62 | 1.82 ± 1.10 | 16.68 ± 5.98 |
| 4 | Post Treatment | 0.77 ± 1.26 | 19.44 ± 10.81 | 0.9 ± 1.73 | 7.10 ± 4.09 |
| 5 | H pylori Gastritis with atrophy | 1.50 ± 2.09 | 24.2 ± 11.75 | 1.39 ± 1.68 | 8.08 ± 4.9 |
| 6 | H pylori Gastritis with IM | 4.7 ± 4.99 | 40.06 ± 17.91 | 1.77 ± 3.16 | 15.70 ± 7.54 |
| 7 | H pylori Gastritis with Dysplasia | 22.16 ± 16.45 | 48.65 ± 16.63 | 4.98 ± 9.62 | 25.24 ± 10.47 |
| 8 | Gastric Adenocarcinoma | | | | 38.60 ± 16.65 |

Our data shows significant increase in PI in CAG without *H.pylori* with a similar expansion of proliferation compartment as compared to normal controls. However, there was significant difference in PI between *H.pylori* positive CAG and *H.pylori* negative CAG. These observations suggest that inflammation increases proliferation both in *H.pylori* positive and *H.pylori* negative CAG as compared to normal control, but *H.pylori* infection has a role in further augmentation of the proliferative response.

Bechi et al^[11] using ³H tritiated thymidine labelling found higher labelling index in *H.pylori* positive CAG biopsies compared to *H.pylori* negative CAG biopsies, however, the difference was not statistically significant. The result of the present study as well as the study by Lynch et al^[22] are contrary to the above. This difference may be due to the different methods used to evaluate cell proliferation. ³H thymidine labelling quantitates only the S phase fraction whereas ki67 quantitates growth fraction (all cycling cells in a population). It is apparent that increased cell turnover with a proportionate shortening of all phases of the cell cycle will not be reflected by a higher S phase fraction. Hence ki67 labelling may be considered a better proliferation marker than ³H thymidine labelling. Therefore, our findings along with those of Lynch et al^[22] support an association of *H.pylori* infection with both increased epithelial cell proliferative activity and expansion of the mucosal proliferative compartment.

In the present study, PI in antral biopsies showing atrophy was also quantitated. PI (combined and neck) was higher in areas of atrophy as compared to normal biopsies. The PI of foveolae and gland region was also higher in atrophic areas when compared to the normal but the difference was not statistically significant. However, the PI of atrophic region was statistically less than that of *H.pylori*

positive CAG. These observations suggest that chronic inflammation in atrophic areas is responsible for persistent increased proliferation.

In the present study, PI in areas of IM was found to be higher in all topographical areas (foveolae, neck and gland) of mucosa as compared to normal controls and was comparable to that of *H.pylori* positive CAG. Similar results were shown by Tseng et al^[16]. Ierardi et al^[25] demonstrated that hyperproliferation associated with IM is not reversed by *H.pylori* eradication, suggesting that proliferation of the metaplastic cells is no longer dependent on bacterial factors.

We also studied antral biopsies showing dysplasia and the PI (combined and topographical) was significantly higher in these dysplastic regions when compared with normal control and *H.pylori* positive CAG. However, PI was statistically increased in the foveolar region only when compared with *H.pylori* positive CAG and areas of IM respectively. This suggests that as there is progression from IM to dysplasia, the proliferative compartment shifts to the foveolar region (superficialisation of the proliferative compartment). We also studied PI in gastric adenocarcinomas of intestinal type and found higher PI in the carcinomatous areas as compared to NC and other groups. The combined PI in carcinoma was significantly higher than *H.pylori* positive CAG, atrophy, IM and dysplasia.

Our data to assess the response of proliferation to *H.pylori* eradication therapy performed on post treatment biopsies showed significant decrease in PI as compared to pretreatment biopsies. However, proliferation in post treatment biopsies was as high as *H.pylori* negative CAG, thereby suggesting a lowering of proliferation after eradication of *H.pylori*. The continuing chronic

inflammation that persists in post treatment biopsies could be responsible for the PI higher than normal controls. Lynch et al^[22] and Brenes et al^[10] have shown similar results.

Conclusion

In summary, *H.pylori* associated gastric carcinoma is a multistep process and evolves through the stages of chronic active gastritis to atrophy, intestinal metaplasia and dysplasia. The present study has shown that *H.pylori* infection is associated with increased proliferation in all regions of gastric mucosa as compared to normal controls. As there is progression from CAG to IM, dysplasia and carcinoma, cell proliferation increases progressively. Eradication of *H.pylori* markedly reduces cell proliferation but does not bring it down to normal levels, suggesting also a role of inflammation in proliferation.

Funding

None

Competing Interests

None

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