Iraqi JMS

Published by Al-Nahrain College of Medicine
ISSN 1681-6579
Email: Iraqi_jms_alnahrain@yahoo.com
http://www. colmed-nahrain.edu.iq/

PCNA Expression in cagA Strain H. pylori Gastritis: Immunohistochemical and *Insitu* Hybridization Study

Thair W Ali[1] *MBChB, PhD*, Ahmed Kh Mahdi[1] *MBChB, MSc*, Hussam H Ali[1] *MBChB, FICMS*, Hassan A Hassan[2] *MBChB, FRCS*

[1]Dept. of Pathology and Forensic Medicine, [2]Dept. of Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Abstract

Background Carriage of *Helicobacter Pylori (H. Pylori)* in the human stomach is associated with increased risk of peptic ulcer disease, distal gastric adenocarcinoma and gastric B-cell mucosa associated lymphoid tissue lymphoma. Several studies have shown increased evidence of increased cell proliferation in the gastric mucosa both in human carrying *H. Pylori, and* animal model of *H. Pylori* infection.

Objective To study the immunohistochemical expression of Proliferating cell nuclear antigen (*PCNA*), as a proliferative marker in the gastric mucosa of patients infected with *CagA Helicobacter Pylori* demonstrated by *insitu* hybridization method.

Methods Gastric antrum and corpus biopsies from 99 patients with dyspeptic symptoms (50 men, 49 women, and median age 40) were analyzed for *H. pylori*, presence of chronic inflammation, intestinal metaplasia, and atrophy according to updated Sydney system. *Insitu* hybridization technique was done to detect cagA *H. pylori*. Immunostaining for PCNA (Avidin- Biotin method) was performed on paraffin embedded tissue specimens.

Results Forty four patients (44.44%) had *H. Pylori* cagA positive strain. Atrophy of gastric mucosa was present in 14 (14.14 %) patients. Intestinal metaplasia was present in 8 (8.08%) patients. The frequency of atrophy was significantly higher in cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (p=0.041). The frequency of intestinal metaplasia was significantly higher in cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (p=0.023). PCNA labeling index (LI) of the gastric glands was significantly higher in presence of atrophic alterations (p <0.001), intestinal metaplasia (p <0.001) and in cagA strain *H. Pylori* positive gastritis (p<0.001).

Conclusion The rates of gastric glandular atrophy, intestinal metaplasia, and epithelial proliferation increase in the presence of *H. Pylori* infection, and are further increased when *H. Pylori* is of cag A strain.

Key words cag A *H. pylori* gastritis, PCNA immunohistochemical expression.

Introduction

Carriage of *Helicobacter Pylori* in the human stomach is associated with increased risk of peptic ulcer disease, distal gastric adenocarcinoma and gastric B-cell mucosa associated lymphoid tissue lymphoma [(1)].

In developed countries, strains of *Helicobacter Pylori* that carry the cag Pathogenesity Island, a 35-40 Kb DNA fragment encoding a series of virulence-related gene associated with an extracellular secretory apparatus, are associated with a greater risk of peptic ulcer and adenocarcinoma than strains that are negative

for cag island [2,3]. Several studies have shown increased evidence of increased cell proliferation in the gastric mucosa both in human carrying *Helicobacter Pylori* [4-10] and animal model of *Helicobacter Pylori* infection [11,12]. After eradication therapy, increased proliferation returns to normal levels, which suggests that *Helicobacter Pylori* or the associated inflammatory response is responsible for the increased proliferation observed [4,6-9].

Proliferating cell nuclear antigen (PCNA) is a 36 KDa intranuclear polypeptide protein whose expression is associated with DNA synthesis and cell proliferation [13,14]. It is a useful immunohistochemical marker of cell proliferation because its expression and distribution correlate with cellular proliferation rate and DNA synthesis [15].

The aim of this study is to study the immunohistochemical expression of PCNA, as a proliferative maker in the gastric mucosa of patients infected with *Helicobacter Pylori* demonstrated by *insitu* hybridization method.

Methods

A total of 99 adult patients presented with dyspeptic symptoms referred to the OGD (oesophagogastroduodenoscopy) unit at Al-Kadhimiya teaching Hospital in Baghdad with an age range of 19-70 years (median 40 years) for upper endoscopy between June 2009 and March 2010 were included. In this study patients who had received anti-ulcer agents or antibiotics for up to two months before the examination and those who had histories of gastric cancer, gastric or duodenal ulcer, or gastric surgery, were excluded. The study was approved by the committee of ethical approval in the College of Medicine, Al-Nahrain University.

Three tissue biopsies were obtained from each patient, two from the antrum and corpus and one from the corpus. Rapid urease test was performed on one of the antral biopsies. The medium used for the test was urea broth. It consists of urea, phenol red indicator and distilled water. One biopsy piece from each sample was inoculated immediately after collection into 1.5ml to 2ml of urea broth. It was incubated at 37°C in the incubator for one and a half hour. The change in color of the broth from pale yellow to deep pink was taken as a positive reaction. The other biopsy specimens were paraffin embedded and processed. One section from each block was stained by H&E to study the histopathological features and grading of gastritis was done according to the updated Sydney system. One section was used for In situ hybridization (ISH) method to identify Cag-A starin *H. pylori*, and one section was stained immunohistochemically for PCNA (Dakocytomation-Mouse monoclonal primary antibody).

Two methods were used to identify *H. Pylori* infection status; rapid urease test and histological sections stained with H&E stain. Patients were considered to be infected with *H. pylori* if one or two of the tests were positive: rapid urease test, or histology. Patients were considered infection free when both of the two tests were negative.

Cell proliferation in gastric epithelium was examined by PCNA labeling indices. The number of positive cells/100 gastric mucosal epithelial cells was counted and was considered to be the proliferation index [16].

In-situ hybridization technique uses biotinylated cDNA probe (for *H. Pylori* cagA gene detection) together with Maxim's ISH detection kit. This complete hybridization and immune detection system, incorporates the biotin-streptavidin amplified technology to provide consistent results and maximum sensitivity to ensure economical and efficient use of the nucleic acid probes. A dark blue signal appears at specific site of the hybridized probe.

Statistical analysis was performed using SPSS 16 and Microsoft Excel 2007. Numeric variables were expressed as mean±SD. Chi-square test

was used to study association between two discrete variables. T-test was used to compare the mean of numeric variables. A P-value of less than 0.05 was considered significant.

Results

Histopathological assessment of gastritis: the assessment was done according to the revised Sydney system [17] (Figure 1).

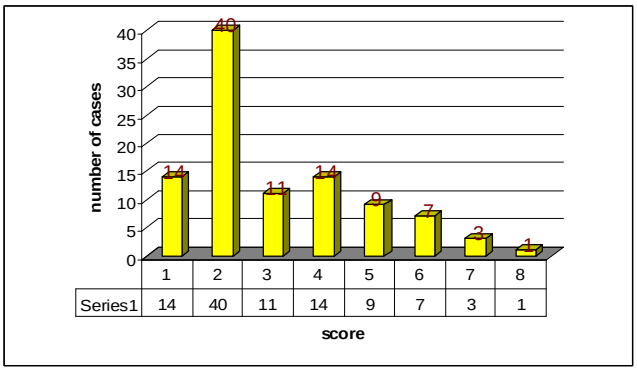

Figure 1. Distribution of cases according to total Sydney score

The results were as follow:

1. Chronic inflammation was mild in 46 (46.46%) patients, moderate in 37 (37.37%) patients and severe in only 16 (16.16%) patients.
2. Active inflammation was present in only 34 out of 99 patients (34.34%), and it was mild in most of the cases (29 patients {29.29%}).
3. Atrophy of gastric mucosa was present in only 14 out of 99 patients (14.14%) and was of mild degree.
4. Intestinal metaplasia was present in 8 out of 99 patients (8.08%), and it was of mild degree.
5. *H. Pylori* infection was present in 69 out of 99 patients (69.69%) (44 were cagA positive), and it was of mild degree in most of the case.

Relation between various histopathological parameters and cagA *H. Pylori* status (cagA versus non cagA):

1. Chronic inflammation and CagA: The degree of chronic inflammation in the presence of cagA strain was significantly higher than that in the absence of cagA strain (mean score 2.11±0.65 versus 1.00±0.00; p<0.001) as shown in table 1.
2. Activity of inflammation and cagA: The activity of inflammation in the presence of cagA strain was significantly higher than that in the absence of cagA strain (mean score 0.90±0.64 versus 0.00±0.00; p<0.001) as shown in table 1.
3. Atrophy and cagA status: The degree of atrophy was significantly higher in cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (0.22±0.42 versus 0.16±0.37; p=0.041). Also, the distribution of atrophy was more frequent among cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (13/44 versus 1/25; p=0.011) as shown in tables 1 & 2.
4. Intestinal metaplasia and cagA: The degree of intestinal metaplasia was significantly higher in cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (0.18±0.39 versus 0.00±0.00; p=0.023). Also, the distribution of intestinal metaplasia was more frequent among cagA *H. Pylori* gastritis than non-cagA *H. Pylori* gastritis (8/44 versus 0/25; p=0.044) as shown in tables 1 & 3.

Table 1. Correlation between various histopathological parameters and cag A H. pylori gastritis (cag A versus non cag A)

Histological parameter Mean score±SD	Cag A (present) No. = 44	Non cag A (absent) No. = 25
Ch. inflammation	2.11±0.65	1.00±0.00*
Activity	0.90±0.64	0.00±0.00**
Atrophy	0.22±0.42	0.16±0.37***
Int. metaplasia	0.18±0.39	0.00±0.00****

*= P< 0.001, ** = P< 0.001, ***& = P< 0.04,**** = P< 0.023

PCNA immunohistochemical expression

PCNA LI of the corpus glands was significantly higher in presence of atrophic alterations (9.71±3.60) as compared to the non-atrophic

mucosa (0.75±1.47) (p <0.001). Also, PCNA LI of the antrum glands was significantly higher in presence of atrophic alterations (13.71±3.60) as compared to the non-atrophic mucosa (2.54±2.99) (p <0.001), as shown in table 4. PCNA LI of the corpus glands was significantly higher in presence of intestinal metaplasia (9.00±5.09) as compared to the non-atrophic mucosa (1.40±2.81) (p <0.001). Also, PCNA LI of the antrum glands was significantly higher in presence of intestinal metaplasia (13.00±5.09) as compared to the non-atrophic mucosa (3.34±4.15) (p <0.001) as shown in table 5.

Table 2. distribution of atrophy according to cagA H. pylori infection

P= 0.011		cagA H. Pylori		
		Positive	Negative	Total
Atrophy	Absent	24	31	55
	Present	1	13	14
	Total	25	44	69

Table 3. The distribution of Intestinal metaplasia according to Cag A H. pylori infection

P= 0.044		cag A H. Pylori		
		Positive	Negative	Total
Intestinal Metaplasia	Absent	25	36	61
	Present	0	8	8
	Total	25	44	69

The PCNA LI in the gastric antrum mucosa was significantly higher in cagA strain *H. Pylori* positive gastritis than cagA strain *H. Pylori* negative gastritis (7.79±4.47 and 2.6±3.51 respectively; p<0.001). The PCNA LI in the gastric corpus mucosa was significantly higher in cagA strain *H. Pylori* positive gastritis than cagA strain *H. Pylori* negative gastritis (4.00±4.52and 0.96+2.18 respectively; p<0.001) as shown in table 6.

Table 4. Comparison of PCNA LI between atrophic and non-atrophic mucosa

	Atrophy	No.	Mean±SD	P value
PCNA Corpus	Present	14	9.71±3.60	<0.001
	Absent	85	0.75±1.47	
PCNA antrum	Present	14	13.71±3.60	<0.001
	Absent	85	2.54±2.99	

Table 5. Comparison of PCNA LI in the presence and absence of intestinal metaplasia

	Meta-plasia	No.	Mean±SD	P value
PCNA Corpus	Present	8	9.00±5.09	<0.001
	Absent	91	1.40±2.81	
PCNA antrum	Present	8	13.00±5.09	<0.001
	Absent	91	3.34±4.15	

Table 6. Comparison between corpus and antrum mucosa in regard to PCNA LI

	Cag A	No.	Mean±SD	P value
PCNA LI Antrum	Positive	44	7.79±4.73	<0.001
	Negative	25	2.60±3.51	
PCNA LI corpus	Positive	44	4.00±4.52	<0.001
	Negative	25	0.96±2.18	

Discussion

The sequence of events that have been suggested in the development of gastric carcinoma is chronic inflammation, mucosal atrophy, intestinal metaplasia, dysplasia and carcinoma [16]. The incidence of the precancerous lesions (atrophy and intestinal metaplsia) is variable in different studies. The percentage of atrophy, in those studies, ranged from 9 to 15 % [18-20]; while the percentage of intestinal metaplasia ranged from 35 to 42 % [18-20]. In the

current study the percentages of intestinal metaplasia and atrophy were 8.8% and 14.14% respectively. Several studies have shown a significant positive association between *H. pylori* infection and development of gastric atrophy [21-24], and this finding is in accordance with the result of the current study. Other studies claimed that infection with *H. pylori* is responsible for higher rates of intestinal metaplasia [21,24-26].

Table 7. Correlation between Sydney score and PCNA expression

Histological parameter	PCNA antrum		PCNA corpus	
Chronic inflammation	r *P*	0.401 <0.001	r *p*	0.392 <0.001
Activity	r *P*	0.556 <0.001	r *P*	0.444 <0.001
Atrophy	r *P*	0.787 <0.001	r *P*	0.856 <0.001
Intestinal metaplasia	r *P*	0.532 <0.001	r *P*	0.567 <0.001
H. pylori score	r *P*	0.550 <0.001	r *P*	0.365 <0.001

Again this finding is in accordance with the result of the current study. Some studies concluded that cag A strain is the main pathogen behind the higher rates of gastric atrophy and atrophic gastritis [21]; which again supports the result of the current study. Also those studies had attributed the higher rates of intestinal metaplasia to cag A strain [21]. This is also in accordance with the finding of the current study. All of the eight cases of intestinal metaplasia were seen in chronic atrophic gastritis, and no intestinal metaplasia was present in chronic non-atrophic gastritis. According to these data, it can be concluded that *H. pylori* existence is an important factor for the development of atrophy and that atrophy can cause intestinal metaplasia. This finding is in accordance with Derya *et al* [16].

PCNA:

The current study has revealed that severity of gastritis and presence of *H. pylori* has different impacts on gastric epithelial cell proliferation. A significantly higher proliferation activity was established in the gastric mucosa in atrophy. These findings are in accordance with some other studies [23,27], which suggest higher proliferative activity of epithelial cells of gastric mucosa in the presence of atrophy.

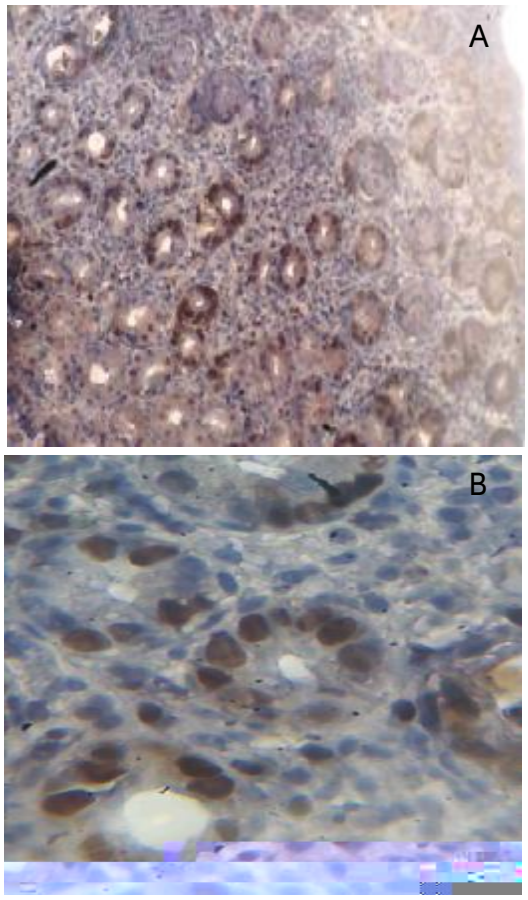

Figure 2. *A*. Positive immunohistochemical expression of PCNA (10X). *B*. Positive immunohistochemical expression of PCNA (40X).

There are data from other authors, which show enhancement of proliferation index with increasing degrees of gastritis both in the antrum and in corpus mucosa [28, 29]. Our data are in concordance with the study of Lynch et al. who have shown also a strong correlation between

epithelial proliferation and chronic inflammatory cell infiltrate [10].

Increased proliferative activity in the atrophic gastric mucosa suggests that the gland cell populations maintain an active cell turnover despite reduction in cellular mass as established through grading criteria.

Some studies showed a positive correlation between the severity of chronic inflammation and the expression of proliferative marker (PCNA) [30]. This is in accordance with findings of the current study.

Steven *et al* showed an increased rate of gastric epithelial cell proliferation in the presence of *H. pylori* infection than in the absence of infection; and that the rate is further increased in the presence of cag A stain *H. pylori* [31]. These data again are in accordance with the results of the current study. Indeed an increase in mucosal cell proliferation increases the likelihood of a neoplastic clone of epithelial cells emerging where there is chronic epithelial cell injury associated with *H. pylori* gastritis and in this way may play a part in gastric carcinogenesis.

Conclusions

The rates of gastric glandular atrophy, intestinal metaplasia, and epithelial proliferation increase in the presence of *H. pylori* infection, and are further increased when *H. pylori* is of cag A strain.

References

1. Parsonnet J. *Helicobacter pylori*. *Infect Dis Clin North Am* 1998; 12: 185-197.
2. Blaser MJ, Perez-Perez GI, Kleanthous H. Infection with *Helicobacter pylori* strains possessing *cag*A is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; 55: 2111-2115.
3. Atherton JC. *H. pylori* virulence factors. *Br Med Bull* 1998; 54: 105-120.
4. Correa P, Ruiz B, Shi TY, et al. *Helicobacter pylori* and nucleolar organizer regions in the gastric antral mucosa. *Am J Clin Pathol* 1994, 10: 656-660.
5. Havard TJ, Sarsfield P, Wortherspoon AC, et al. Increased gastric epithelial cell proliferation in *Helicobacter pylori* associated follicular gastritis. *J Clin Pathol* 1996; 49: 68-71.
6. Fraser AG, Sim R, Sankey EA, et al. Effect of eradication of *Helicobacter pylori* on gastric epithelial cell proliferation. *Aliment Pharmacol Ther* 1994; 8: 167-173.
7. Brenes F, Ruiz B, Correa P, et al. *Helicobacter pylori* causes hyperproliferation of the gastric epithelium: pre- and post-eradication indices of proliferating cell nuclear antigen. *Am J Gastroenterol* 1993; 88: 1870-1875.
8. Lynch DAF, Clarke AMT, Jackson P, et al. Cell proliferation in *Helicobacter pylori* associated gastritis and the effect of eradication therapy. *Gut* 1995; 36: 346-350.
9. Cahill RJ, Xia H, Kilgallen C, et al. Effect of eradication of *Helicobacter pylori* infection on gastric epithelial cell proliferation. *Dig Dis Sci* 1995; 40: 1627-1631.
10. Lynch DAF, Mapstone NP, Clarke AMT, et al. Correlation between epithelial cell proliferation and histological grading in gastric mucosa. *J Clin Pathol* 1999; 52: 367-371.
11. Yu J, Russell RM, Salomon RN, et al. Effect of *Helicobacter* mustelae infection on ferret gastric epithelial cell proliferation. *Carcinogenesis (Lond.)* 1995; 16: 1927-1931.
12. Fox JG, Dangler CA, Taylor NS, et al. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances *Helicobacter pylori* colonization in C57BL/6 mice. *Cancer Res* 1999; 59: 4823-4828.
13. Narita T, Funahashi H, Satoh Y, et al. Proliferating cell nuclear antigen immunostaining in breast cancer and its relation to prognosis. *Jpn J Clin Oncol* 1993; 23(1): 20-25.
14. Shin DM, Vararud N, Ro JY. Sequential increases in proliferative cell nuclear antigen expression in head and neck tumorigenesis: A potential biomarker. *J Natl Cancer Inst* 1993; 85: 971-978.
15. Zuber M, Tan EM, Ryoji M, et al. Involvement of proliferating cell nuclear antigen (cyclin) in DNA replication in living cells. *Mol Cell Biol* 1989; 9: 57-66.
16. Göral DV, Yilmaz F, Kara IH. The relation of Helicobacter pylori with intestinal metaplasia, gastric atrophy and BCL-2. *Turk J Gastroenterol* 2004; 15(3): 149-155.
17. Dixon MF, Genta RM, Yardley JH, et al. Classification & Grading of gastritis: the updated Sydney system, international workshop on the histopathology of gastritis, Houston. *Am J Surg Pathol* 1996; 20: 1161-1181.

18. Wyatt JI, Shallcross TM, Crabtree JE, et al. Helicobacter pylori, gastritis, and peptic ulceration in the elderly. *Jr Clin Pathol* 1992; 45: 1070-1074.
19. Iijima K, Abe Y, Kikuchi R, et al. Serum biomarker tests are useful in delineating between patients with gastric atrophy and normal, healthy stomach. *World J Gastroenterol* 2009; 15(7): 853-9.
20. Tarkhashvili N, Beriashvili R, Chakvetadze N, et al. *Helicobacter pylori* Infection in Patients Undergoing Upper Endoscopy, Republic of Georgia. *Emerg Infect Dis* 2009; 15(3): 504-505.
21. Jakiæ-Razumoviæ J, Tentor D, Kušec V, et al. Histopathological Features of Gastritis before and after Treatment for *Helicobacter pylori. Croat Med J* 2000; 41: 159-162.
22. Kuipers EJ, Lundell L, Klinkenberg-Knol EC, et al. Atrophic gastritis and *H. pylori* gastritis in patients with reflux esophagitis treated with omeprazole or fundoplication. *N Eng J Med* 1996; 334: 1018-1022.
23. Lipkin M, Correa P, Mikol YB, et al. Proliferative and antigenic modifications in human epithelial cells in chronic atrophic gastritis. *J Natl Cancer Inst* 1985; 75: 613-9.
24. Asaka M, Sugiyama T, Nobuta A, et al. Atrophic gastritis and intestinal metaplasia in Japan: results of a large multicenter study. *Helicobacter* 2001; 6: 294-9.
25. Kuipers EJ, Pérez-Pérez GI, Meuwissen SGM, et al. *Helicobacter pylori* and Atrophic Gastritis: Importance of the cagA Status. *J Nat Cancer Inst* 1995; 87(23): 1777-1780.
26. Kenji O, Mitsuo O, Hiroshi M, et al. Association of Helicobacter pylori infection with atrophic gastritis and intestinal metaplasia. *J Gastroenterol Hepatol* 2000; 15(10): 1105.
27. Vorobjova T, Hürlimann S, Zimmermann A, et al. *Helicobacter pylori* gastritis: glandular proliferation and homeostasis differ between gastric antrum and corpus. *Acta Medica Lituanica* 2005; 12(3): 18-27.
28. Hart HO, Johansen AA, Larsen JK, et al. Cell proliferation in normal and diseased gastric mucosa. Autoradiography after *in vitro* continuous labeling with tritiated thymidine. *Acta Path Microbial Scand* 1979; Sect. A. 87: 217–22.
29. Chow KW, Bank S, Ahn J, et al. *Helicobacter pylori* infection does not increase gastric antrum mucosa cell proliferation. *Am J Gastroenterol* 1995; 90: 64-6.
30. Akbulut M, Demirkan N, Düzcan E. PCNA expression in chronic gastritis due to Helicobacter pylori. *Turk J Gastroenterol* June 2006; 17(2): 84-9.
31. Moss SF, Sordillo EM, Abdalla AM, et al. Increased Gastric Epithelial Cell Apoptosis Associated with Colonization with *cagA*1 *Helicobacter pylori* Strains. *Cancer Res*. Feb. 2001;61: 1406-1411.

Corresponding to Dr. Ahmed Kh Mahdi
E-mail: ahmad_khm2000@yahoo.com
Received 26th Dec. 2011: accepted 10th Jan. 2012.