# Bcl-2 Expression in CagA Strain H. Pylori Gastritis (Immunohistochemical and Insitu Hybridization Study)

**Hussam Hasson Ali[*], Hassan Ahmad Hassan [**], Bashar A. Abdul Hassan [***], Thair Wali Ali [****]**


## ABSTRACT:

**BACKGROUND:**

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.

**OBJECTIVE:**

To study the immunohistochemical expression of bcl-2, as apoptosis makers in the gastric mucosa of patients infected with cagA Helicobacter Pylori demonstrated by insitu hybridization method.

**PATIENTS MATERIALS AND METHODS:**

Gastric antrum biopsies from 99 patients presented with dyspeptic symptoms (50 men, 49 women, median age 40) were analysed for the presence of *H. pylori*, and were classified according to updated Sydney system. Insitu hybridization technique was done to detect cagA *H. pylori*. Immunohistochemical expression of bcl-2 using (Avidin- Biotin method) was performed on paraffin embedded biopsy specimens.

**RESULTS:**

Forty four patients (44.44%) had H. pylori cagA positive starin. Atrophy of gastric mucosa was present in 14 (14.14 %) patients. Intestinal metaplasia was present in 8 (8.08%) patients. The frequency of atrophy and intestinal metaplasia were significantly higher in cagA H. pylori gastritis than non-cagA H. pylori gastritis ($p=0.023$ and 0.041respectively). Bcl2 expression was not significantly higher in H. pylori gastritis than non-H. pylori gastritis ($p= 0.101$). Bcl2 expression was significantly higher in the presence of atrophy ($p<0.001$). Bcl2 expression was significantly higher in the presence of intestinal metaplasia ($p<0.001$).

**CONCLUSION:**

The rate of apoptosis decreases when lesions (gastric atrophy and intestinal metaplasia) are present.


***KEY WORDS***: cag A *H. pylori* gastritis, Bcl2 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 adeocarcinoma than strains that are negative for cag island. [(2,3)]

Because of the increasing realization that cell turn over is dependent not only on proliferation but also on apoptotic cell loss [(4)], and because it is now appreciated that many pathogenic bacteria are capable of interacting with the apoptotic program of epithelial cells [(5)], the effect of *Helicobacter Pylori* on gastric epithelial cells apoptosis also has been recently investigated. The presence of *Helicobacter Pylori* has been associated with a 2-5 fold increase in gastric epithelial apoptosis in vivo that returns to normal levels after eradication of the organism in most studies. [(6, 7)]

However in other studies, apoptosis was reported as unchanged [(8)] or even decreased in the presence of *Helicobacter Pylori*.[(9)]


* Department of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

** Department of General Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq

*** Department of General Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq

**** Department of Pathology and Forensic Medicine, College of Medicine, Al-Nahrain University, Baghdad, Iraq

## AIMS OF THE STUDY:

To study the immunohistochemical expression of bcl-2, as apoptosis makers in the gastric mucosa of patients infected with cagA *Helicobacter Pylori* demonstrated by insitu hybridization method.

## PATIENTS MATERIALS AND METHODS:

The study was prospectively designed. A total of 150 adult patients presented with dyspeptic symptoms referred to the OGD (esophagogastroduodenoscopy) unit at Al-Kadhimiya teaching Hospital at 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.

Fifty one patients were excluded from the study because the biopsy material obtained during OGD had been insufficient for the application of immunohistochemical and in situ hybridization studies. Three tissue biopsies were obtained, two from the antrum and one from the corpus. Rapid urease test was performed on one of the antral biopsies. 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 method to identify Cag-A starin *H. pylori* one section was stained Immunohistochemically for bcl-2.

Two methods were used to identify H. pylori infection status; rapid urease test and histological sections stained with H&E stain (fig.1 D). Patients were considered to be infected with *H pylori* if one or two of the tests was positive : rapid urease test, or histology. Patients were considered infection free when both of the two tests were negative.

Monoclonal Mouse Anti-Rat bcl-2, is intended for laboratory use to identify qualitatively by light microscopy bcl-2 positive cells in normal and Neoplastic tissues using immunohistochemical (IHC) test methods. The Immunophosphatase secondary detection system was Dako Cytomation LSAB2 System-HRP, Code K0673. The DNA Probe used in insitu hybridization was Biotinylated DNA probe for *H. pylori* cagA gene. The DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech).

Immunohistochemical staining for bcl-2 was assessed as positive or negative cytoplasmic staining (fig.1A). Positive control is the lymphoid tissue in reactive follicular hyperplasia (fig.1 C). Technical negative control was obtained by omission of primary antibody.

In-situ hybridization technique uses biotinylated cDNA probe (for H. pylori cagA gene detection) together with Maxim's ISH detection kit. a dark blue signal appears at specific site of the hybridized probe (fig.1 E).

Statistical analysis was performed using SPSS 16 and Microsoft Excel 2007. Continuous numeric variables were expressed as mean $\pm$ SD. Chi-square test was used to compare between two discrete variables. T-test and ANOVA were used to compare the mean of numeric variables. Pearson correlation test was used to assess correlation between two continuous numeric variables. A P-value of less than 0.05 was considered significant.

## RESULTS:

Association between various histopathological parameters and cagA H. pylori status (cagA versus non cagA):

Sixty nine patients were positive for *H. pylori* and 30 patients were negative for *H. pylori*. From those 69 patients, 44 patients were positive for cag A strain *H. pylori* as detected by in situ hybridization technique. Application of updated Sydney system on those 69 patients, who were positive for *H. pylori*, revealed the following results:

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 $2.11\pm0.65$ versus $1.00\pm0.00$; $p<0.001$) (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 $0.90\pm0.64$ versus $0.00\pm0.00$; $p<0.001$) (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 ($0.22\pm0.42$ versus $0.16\pm0.37$; $p=0.041$) (Table 1). Also, the distribution of atrophy was more frequent among cagA H. pylori gastritis than non-cagA H. pylori gastritis (13/44 versus 1/25; $p=0.011$).

4. Intestinal metaplasia and cagA

The degree of intestinal metaplasia (fig.1 B) was significantly higher in cagA H. pylori gastritis than non-cagA H. pylori gastritis ($0.18\pm0.39$ versus $0.00\pm0.00$; $p=0.023$) (table 1). 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).

Immunohistochemical expression of bcl2

The relation between bcl2 expression and cag A H. pylori infection

Bcl2 expression was significantly higher in cag A H. pylori gastritis than non-cagA H. pylori gastritis (8/44 versus 0/25; p= 0.044) (table 2, fig.1A).

**Table 1: Relation between various histopathological parameters and cagA H. pylori status (cagA versus non cagA).**

| Histological parameter | | cagA H. pylori | Non-cagA H. pylori | p-value |
|---|---|---|---|---|
| Chronic inflammation | n | 44 | 25 | <0.001 |
| | Mean score ±SD | 2.11±0.65 | 1.00±0.00 | |
| Activity | n | 44 | 25 | <0.001 |
| | Mean score ±SD | 0.90±0.64 | 0.00±0.00 | |
| Atrophy | n | 44 | 25 | 0.041 |
| | Mean score ±SD | 0.22±0.42 | 0.16±0.37 | |
| Intestinal metaplasia | n | 44 | 25 | 0.023 |
| | Mean score ±SD | 0.18±0.39 | 0.00±0.00 | |

**Table 2: The correlation between bcl2 expression and H. pylori infection.**

| P= 0.044 | | Cag A H. pylori | | Total |
|---|---|---|---|---|
| | | Negative | Positive | |
| BCL 2 | Negative | 25 | 36 | 61 |
| | Positive | 0 | 8 | 8 |
| | Total | 25 | 44 | 69 |

**Table 3: The relation between bcl2 expression and atrophy.**

| P <0.001 | | Atrophy | | Total |
|---|---|---|---|---|
| | | Absent | Present | |
| BCL 2 | negative | 83 | 8 | 91 |
| | Positive | 2 | 6 | 8 |
| | Total | 85 | 14 | 99 |

**Table 4: The relation between bcl2 expression and intestinal metaplasia.**

| P <0.001 | | Intestinal metaplasia | | Total |
|---|---|---|---|---|
| | | Absent | Present | |
| BCL 2 | negative | 88 | 3 | 91 |
| | Positive | 3 | 5 | 8 |
| | Total | 91 | 8 | 99 |

The relation between bcl2 expression and atrophyWhen all the cases were considered, H. pylori positive and negative, bcl2 expression was significantly higher in the presence of atrophy in comparison with absence of atrophy (6/8 in comparison with 2/83; p<0.001) (table 3).The relation between bcl2 expression and intestinal metaplasiaWhen all the cases were considered, H. pylori positive and negative, bcl2 expression was significantly higher in the presence of intestinal metaplasia in comparison with absence of intestinal metaplasia (6/8 in comparison with 2/83;p<0.001)(table4).

## DISCUSSION:

The balance between cell proliferation and cell loss indicate that Bcl-2 is upregulated in gastric premalignant lesions and downregulated after malignant change. [10,11]Maor-Kendler et al. [12] found that bcl-2, which blocks apoptosis, increases in atrophic gastritis; however, they could not find a direct relation of Hp in this situation, and reported that bcl-2 positivity arises from atrophy. Jorge et al. [13] performed antral biopsy in 57 patients with chronic gastritis, studied the relation of H pylori with bcl-2 and found that H pylori positivity directly increases the bcl-2 expression. II Ju Choi et al. [14] reported that H pylori directly increases

the expression of anti-apoptotic bcl-2, as well as causes apoptosis. Yang et al. (2003) [15] found that H pylori causes apoptosis by decreasing the expression of bcl-2. Derya et al described a positive non-significant correlation between bcl2 expression and degree of H .pylori infection; and a significant positive correlation between atrophy and bcl2 expression [16]. These data are in accordance with the data obtained from the present study.This result illustrates that H pylori may increases bcl-2 expression. But the increment may be due to atrophy and not to H pylori. This result supports the suggestion of Maor-Kendler et al. [12] that increased bcl-2 expression is related to atrophy but not associated directly with H pylori. Nevertheless, it does not support the studies of Jorge et al. [13] and II Ju Choi et al. [14], who suggested that H pylori increases bcl-2 directly, nor of Yang et al. [15] who suggested that H pylori decreases the expression of bcl-2.The present study showed a significant positive correlation between bcl2 expression and intestinal metaplasia; and this is similar to the results of other studies [16,17]. In the present study, the proportion of cases with intestinal metaplasia and positive bcl-2 expression was higher than the proportion of cases with both mucosal atrophy and positive bcl-2 expression. The greater bcl-2 expression in IM compared to atrophy may support the study of Heng Jun et al. [17] and may suggest that bcl-2 expression increases while advancing to gastric cancer.

## CONCLUSION:

The rate of apoptosis decreases when lesions (gastric atrophy and intestinal metaplasia) are present.

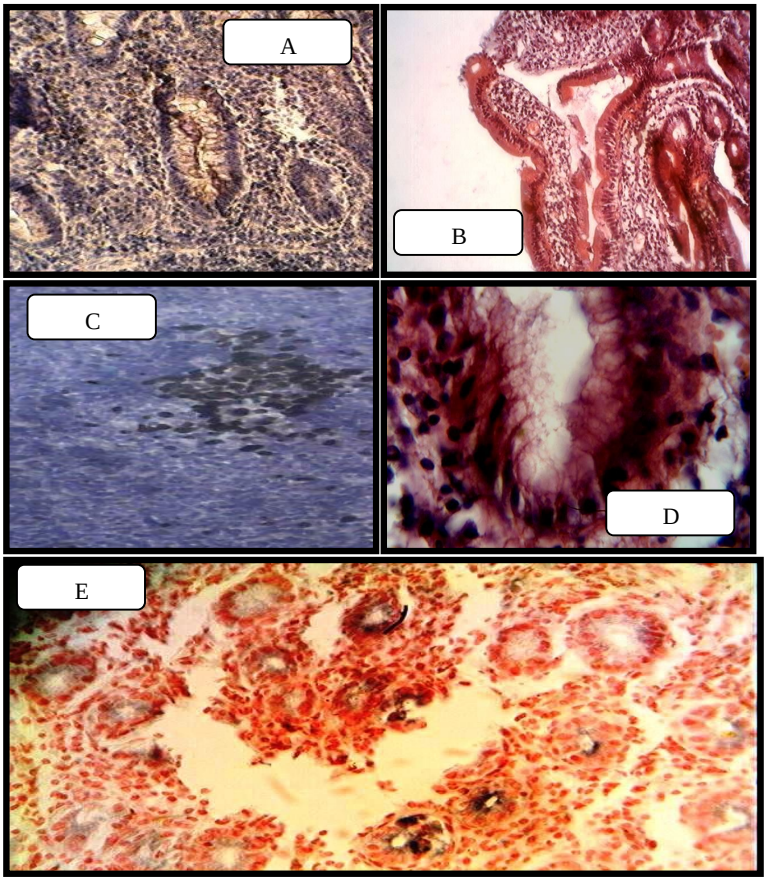


**Figure 1 A: positive bcle2 expression (cytoplasmic) (20 X and 40 X respectively). B: intestinal metaplasia in gastric mucosa C: bcl2 positive control (follicular hyperplasia) (brown cytoplasmic staining) (20X). D*: H. pylori* infection of moderate score (H&E stain 100X). E: Demonstration of cagA strain H. pylori by in situ hybridization technique (20 X).**

## REFERENCES:


1. Parsonnet, J.. *Helicobacter pylori*. Infect. Dis. Clin. North Am., 1998;12:185–97.
2. Blaser, M. J., Perez-Perez, G. I., 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: 2111–15.
3. Atherton, J. C.. *H. pylori* virulence factors. Br. Med. Bull., 1998;54*:* 105–20.
4. Hall, P. A., Coates, P. J., Ansari, B., and Hopwood, D.. Regulation of cell number inthe mammalian gastrointestinal tract: the importance of apoptosis. J. Cell Sci., 1994;107:3569–77.
5. Zychlinsky, A., and Sansonetti, P.. Apoptosis in bacterial pathogenesis. J. Clin. Investig., 1997;100*:* 493–96.
6. Houghton, J., Korah, R. M., Condon, M. R., and Kim, K. H.. Apoptosis in *Helicobacterpylori*-associated gastric and duodenal ulcer disease is mediated via the Fasantigen pathway. Dig. Dis. Sci., 1999;44 465–78.
7. Rudi, J., Kuck, D., Strand, S., von Herbay, A., Mariani, S. M., Krammer, P. H., Galle, P. R., and Stremmel, W.. Involvement of the CD95 (APO-1/Fas) receptor and ligand system in *Helicobacter pylori*-induced gastric epithelial apoptosis. J. Clin. Investig.1998;102*:*1506–14.
8. Anti, M., Armuzzi, A., Iascone, E., Valenti, A., Lippi, M. E., Covino, M., Vecchio, F. M., Pierconti, F., Buzzi, A., Pignataro, G., Bonvicini, F., and Gasbarrini, G. 1998. Epithelial-cell apoptosis and proliferation in *Helicobacter pylori*-related chronic gastritis. Ital. J. Gastroenterol. Hepatol., *30:* 153–159
9. Hirasawa, R., Tatsuta, M., Iishi, H., Yano, H., Baba, M., Uedo, N., and Sakai, N. Increase in apoptosis and decrease in ornithine decarboxylase activity of the gastric mucosa in patients with atrophic gastritis and gastric ulcer after successful eradication of *Helicobacter pylori*. Am. J. Gastroenterol. 1999;94*:*2398–2402.
10. Scopa CD, Vagianos C, Kardamakis D, et al. 2001; Bcl-2/bax ratio as a predictive marker for therapeutic response to radiotherapy in patients with rectal carcer. Appl Immunohistochem Mol Morphol 9:329-334.
11. Nakamura T, Nomura S, Skai T.; Expression of bcl-2 oncoprotein in gastrointestinal and uterine carcinomas and their precursor lesions. Hum Pathol 1997;28:309-15.
12. Maor-Kendler Y, Gabay G, Bernheim J, et al.; Expression of bcl-2 in autoimmune and Helicobacter pylori-associated atrophic gastritis. Dig Dis Sci 1999;44 : 680-5.
13. Jorge O, Cuello Carrion FD, Jorge A, Ciocca DR.; Helicobacter pylori infection affects the expression of PCNA, p53, cerbB-2 and Bcl-2 in the human gastric mucosa. Rev Esp Enferm Dig 2003;95:97-104. 89-96.
14. Ju Choi, Joo Sung K, Jung Mogg K, et al.; Effect of inhibition of extracellular signal-regulated kinase 1 and 2 pathway on apoptosis and bcl-2 expression in Helicobacter pylori-infected AGS cells. Infection and Immunity 2003;71: 830-7.
15. Yang Y, Deng CS, Peng JZ, et al.; Effect of Helicobacter pylori on apoptosis and apoptosis related genes in gastric cancer cells. Mol Pathol 2003;56:19-24.W.
16. Derya Vedat GÖRAL1, Fahri YILMAZ2, İsmail Hamdi KARA3;; The relation of Helicobacter pylori with intestinal metaplasia, gastric atrophy and BCL-2 Turk J Gastroenterol 2004;15:149-55.
17. Heng Jun G, Lian Z, Jian Feng B, et al.; Multiple genetic alterations and behavior of cellular biology in gastric cancer and other gastric mucosal lesions: H. pylori infection, histological types and staging. World J Gastroenterol 2000;6:848-54.