Molecular and Immunopathological Role of Nuclear Factor K B Detected By Insitu Hybridization in Pathogenesis of Chronic Atrophic Gastritis in Iraqi Patients

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Abstract

Chronic Atrophic gastritis (CAG) is a histopathological entity characterized by chronic inflammation of the gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium and fibrous tissue. Atrophy of the gastric mucosa is the endpoint of chronic processes, such as chronic gastritis associated with *Helicobacter pylori* infection. This study designed to determine the molecular role of NFkB signaling on Fas receptor mediated apoptosis in *H.pylori* associated CAG cases.

Forty five patients suffering from CAG that proved via endoscopical and histopathological examination were eligible for this study. Males represent (73.33%) and the rest (26.67%) were females with a mean age of 39.26 years .twenty apparently healthy volunteers (10 male and 10 female) with the mean age 38 years and age range (23-58) years were enrolled as control group.

Multiple mucosal biopsy specimens were taken from the inflamed area of gastric mucosa for rapid urease test other biopsy specimens were fixed with 10% buffered formalin for preparation of paraffin embedded tissue blocks to prepare slides for histological examination using haematoxylin-eosin stain to evaluate the grades of Polymorphonuclear leukocytes(PMNs) and lymphocytes infiltration . Insitu hybridization technique (ISH) using biotinylated long DNA probe for human NF-KB Gene and for human FAS gene as well as *H.pylori* Cag A gene were used for detection of NF-KB and Fas mRNA and Cag A mRNA expression in tissue infiltrated lymphocytes and neutrophils as well as gastric epithelial cells and for detection of *H.pylori* Cag A.

This study explained that there was a statistical significant positive linear relationship between lymphocytes grade and Fas mRNA expression in PMNs and gastric epithelial cells. PMN grade has significant positive linear relationship with each of the following markers: Tissue Fas mRNA expression. Tissue NFkB mRNA expression, lymphocytes NFkB mRNA expression, PMN NFkB mRNA expression, Cag A mRNA expression.

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Lymphocytes Fas mRNA expression has significant positive linear relationship with PMN Fas mRNA expression and Cag A mRNA expression .PMN Fas mRNA expression has significant positive linear relationship with Cag A mRNA expression and negative linear relationship with tissue NFkB mRNA expression . Tissue Fas mRNA expression has significant positive linear relationship with tissue NFkB mRNA expression ; lymphocytes NFkB mRNA expression ; PMN NFkB mRNA expression ;Cag A mRNA expression . Tissue NFKB mRNA expression has significant positive linear relationship with lymphocytes NFkB mRNA expression; PMN NFkB mRNA expression. Lymphocytes NFkB mRNA expression has significant positive linear relationship with PMN NFkB mRNA expression.

This study conclude that NFkB play a vital role in determination of immunopathological and inflammatory events of gastritis that leads finally to development of atrophic changes.

Key words: atrophic gastritis, NFkB, Fas, H.pylori $^{cagA+/urease+,}$ISH

Introduction

Atrophic gastritis is a histopathological entity characterized by chronic inflammation of the gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium and fibrous tissue. Atrophy of the gastric mucosa is the endpoint of chronic processes, such as chronic gastritis associated with *Helicobacter pylori* infection, other unidentified environmental factors, and autoimmunity directed against gastric glandular cells.

The single layer of epithelial cells that lines the gastric mucosa is the initial site of interaction between the host and *H. pylori*. Gastric epithelial cells respond to *H. pylori* infection by activating NF-κB (Maeda *et al*,2000; Naumann *et al*,2000)[1,2] , up regulating the expression of a proinflammatory gene program, which includes the chemokine IL-8,cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (Kim *et al*,2000; Kim *et al* 2002)[3,4] , and by finally undergoing apoptosis . These relationships suggest that the signals produced by gastric epithelial cells can influence the inflammatory responses and
apoptosis of gastric epithelial cells following *H. pylori* infection. Many of the genes that are activated in gastrointestinal epithelial cells after bacterial infection are target genes of NF-KB(Jobin *et al*, 2000; MacDonald *et al*, 2000) [5,6].NF-KB is a dimeric transcription factor, which is normally held in the cytoplasm in an inactive state by inhibitory proteins, the IκB kinases (IκB) .

The stimulation of epithelial cells with several pathogens or inflammatory cytokines can induce IκB degradation and subsequently cause the NF-KB complex to migrate to the nucleus and to bind to DNA recognition sites in the regulatory regions of target genes (Elewaut *et al*,1999) [7] .The activation of NF-KB requires the phosphorylation of IκB-α (Ser32 and Ser36) and IκB-β (Ser19 and

Ser23) . This phosphorylation leads to ubiquitination and the 26S proteosome-mediated degradation of IκB-α thereby releasing NF-KB from the complex to enter the nucleus and activate genes .Two cytokine-inducible kinases, IKK_α and IKK_β, have been cloned and characterized (Zandi *et al*,1997) [8] . IKK_α and IKK_β phosphorylate IκB-α at Ser32 and Ser36 in response to proinflammatory cytokines. NF-kB-inducing kinase (NIK) is a novel member of the MEKK family and was found to activate both IKK_α and IKK_β by interacting with adapter proteins associated with receptors for TNF-α and IL-1 (Malinin *et al*,1997) [9]. Recently, it was demonstrated that *H. pylori* could activate NF-KB via a signaling pathway involving IKK and NIK (Maeda *et al*, 2000) [1]. Given that many cell types become more sensitive to TNF-α or *H. pylori* induced apoptosis after the suppression of NF-KB activity (Schmid *et al*, 2000)[10],NF-KB may function antiapoptotically. In contrast, other studies showed that the suppression of NF-KB activation inhibited *H. pylori*-induced apoptosis in gastric epithelial cells (Lim *et al*, 2001) [11]. These conflicting observations indicate that the role of NF-KB in *H.pylori*-induced apoptosis in gastric epithelial cells has not been clarified.

2. Aim of the study:

This study designed to determine the Molecular and immunopathological role of NFkB signaling on Fas receptor mediated apoptosis in chronic atrophic gastritis cases presented with *H.pylori* infection.

Patients Materials and Methods

3.1 Patients

Patients attending the gastroenterology unit of digestive and hepatic diseases teaching hospital in Baghdad were diagnosed as having atrophic gastritis by upper gastrointestinal endoscopy from May 2008 to December 2009 were eligible for this study.

Patients who had received antiulcer agents, antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) in the two months before the examination, the result of rapid urease test was negative were excluded. All patients provided informed consent before endoscopy. Data were collected through direct interview with the patient, and by seeking his/her hospital record as well as previous medical reports.

According to the exclusion criteria forty five patient examined in the gastroenterology unit (*33* male and 12 female, with a mean age of 39.26 years (range 58 years) suffering from chronic atrophic gastritis were chosen. Twenty apparently healthy volunteers (10 male and 10 female) with the mean age 38 years and age range (23-58) years were enrolled as control.

3.2. Materials

A. Sample collection:

Patients were fasted for at least eight hours before endoscopic examination. Endoscopic examination was performed under local pharyngeal anesthesia. Using fiber optic endoscope, multiple mucosal biopsy specimens were taken via sterile standard biopsy forceps from the stomach (inflamed area of gastric mucosa). One biopsy specimens were used for rapid urease test (RUT) for detection of *H.pylori* in tissue sample. Other biopsy specimen was used for gram stain. Last biopsy specimen was fixed with 10% buffered formalin for preparation of paraffin embedded tissue blocks to prepare slides for histological examination using haematoxylin-eosin stain. Biopsy specimens obtained from the antrum were used to evaluate the grades of Polymorphonuclear leukocytes and lymphocytes infiltration and according to the updated Sydney System. The presence of *H.pylori* in tissue section was established by typical appearance on scanning along the mucosal surface and the individual gastric pits.

B. DNA Probe:

i. Biotinylated long DNA probe for human Fas Gene, Cat. No.: IH-60047(fas-6001-B). (Maxim biotech-USA):

ii. Biotinylated long DNA probe for human NF-KB gene, Cat. No. IH-60031) (Maxim biotech-USA).

iii. Biotinylated long DNA probe for H.pylori/ Cag A Gene, Cat. No.: IH-60061(HPY-6001-B) (Maxim biotech-USA).

C. The DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech-USA).

3.3. Methods

A. Biopsy urease test (BUT):

Biopsy specimens are to be placed in 0.5ml of lactated Ringers solution at pH 6.5.transport to laboratory on wet ice. Grind biopsy specimens with a sterile tissue grinder, and place a portion of the ground specimen into the rapid urea medium. As an alternative to this method, the biopsy may be placed directly into the rapid urea medium. At the time of endoscopy, if desired. Submerge the specimen in the rapid urea medium. Incubate at room temperature (15-30 C°) aerobically. Observe at 15-20 minutes and again at one, three, and six hours of incubation for the development of a pink-red or red-violet color. Continue incubation of negative tests for up to 20 hours. Rapid urea medium may be incubated at 35 C°. in order to obtain faster reaction times. (Forbes, *et al.*, 1998)[12] .

A positive reaction is indicated by the appearance of a pink-red to violet color. No color change is indicative of a negative reaction. A strong positive reaction may be determined within minutes of inoculation into the rapid urea medium. (Forbes, *et al.*, 1998) [12].

B. ***Insitu*** **hybridization procedure** (According to Maxim biotech instruction manual (see www. Maxim biotech .com for *insitu* hybridization detection system)[13].

Serial tissue sections were cut *4-6* µm thick and were positioned on positively charged slides. The slides were then heated at 80°C overnight. The tissue sections were deparaffinized by standard methods. The slides were treated with Proteinase K solution and dehydrated. One drop of the Biotinylated long DNA probe for human NF-KB or Fas gene or Cag A for *H.pylori* hybridization solution was placed on the tissue section in oven or heating block at *70°C* for 8-10 minutes to denature the secondary structure of RNA. After

that slides were placed in a humid chamber and incubated at *37°C* for 3-4 hours to allow hybridization of the probe with the target nucleic acid. The slides were soaked in detergent wash at *37°C* until the cover slips fell off, and then treated with RNase A and the conjugate. One to two drops of substrate were placed on tissue section at room temperature for about 10 minutes, or until color development was complete, the latter was monitored by viewing the slides under the microscope. A blue colored precipitate will form at the site of the probe in positive cells. Slides were then counterstained using nuclear fast red and sections were mounted with a permanent-mounting medium (DPX). Finally the examination and scoring were done under light microscope by a pathologist at power X400 according to the scoring system shown in table I.

Table (I) Scoring system used in Insitu hybridization according to Al- Izzi (2006) [14], khadim (2004)[15]

Marker	*Negative*	*Low*	*Intermediate*	*High*
NFkB mRNA Expression	*< 5%*	*5-25%*	*26-50%*	*>50%*
Fas mRNA Expression				

C. Scoring system for PMNs and lymphocytes according to Mahdavi, (2004)[16]

1. Lymphocyte infiltration:

Grading scale from 0 to 3, based on both lymphocyte and plasma cell infiltration as shown in table II

Table (II): Grading system of Lymphocyte infiltration

Grade of Lymphocyte infiltration	Interpretation

Grade 0	Normal
Grade 1	low inflammation
Grade 2	Moderate inflammation
Grade 3	heavy inflammation

2. Grade of PMN cell infiltration:

Prepared slides of CAG and control cases were scanned for PMN cell infiltration according to the following grading system.

Table (III): Grading system of PMN cell infiltration

Grade of PMN cell Infiltration	Interpretation
Grade 0	none
Grade 1	rare PMN, only in lamina propria (LP)
Grade 2	≤ 1 intraepithelial (IE) PMN /high power field (hpf), i.e. 400X magnification
Grade 3	1-10 intraepithelial (IE) PMN/ (hpf)
Grade 4	≥ 10 IE/hpf
Grade 5	pit abscess

3.4. Statistical analysis:

Data analysis was performed using the following tests:

1. T-test was used to find out the significance of differences between two groups that composed from continuous variables.

2. Pearson test for correlation was used for non categorical data.

The level of significance was 0.05(two-tail) in all statistical testing; significant of correlations include also 0.01 (two-tail) .The level of confidence limits was 0.095.Statistical analysis was performed using SPSS for windows TM version 14.0, and Microsoft EXCEL for windows 2007.

Results

In this study, forty five patients suffering from chronic atrophic gastritis were chosen as shown in table (1). The minimum age of infected individuals was 18 years while maximum age was 75

years and the mean age was 39.26 years .the maximum age of control group was 58 years and the minimum age 23 years with range of 35 years. Infected males represent (*73.33%)* and the rest (*26.67%*) were females compared with (50%) for each gender in control group as illustrated in table (2).

H.pylori with negative Cag A mRNA expression insitu and positive production of urease was determined in 30(66.7%) Out of 45 patient, while in 15(33.3%) CAG cases *H.pylori* with positive Cag A mRNA expression *insitu* and positive production of urease was detected compared with negative results in all cases of control group as shown in table (3) and figure(1).

Table (4) reveal that grade (3) of tissue infiltrated lymphocytes represent (53.3%) and (46.7%) for grade (2) compared with absence of lymphocytic infiltration among control group. As shown in table (5) high score of lymphocytes NFkB mRNA expression was detected in (53.33%) of CAG cases compared with (33.34%) for intermediate score and (13.34%) for low score .low score of lymphocytes NFkB mRNA expression was detected equally in (6.66%) among grade (2) and (3). Intermediate lymphocytes NFkB mRNA expression was detected in (20%) among grade (3) compared with (13.33%) in grade (2) lymphocytes infiltration. High score of lymphocytes NFkB mRNA expression was detected equally in (26.66%) among grade (2) and (3) of lymphocytes infiltration. All these results associated with negative results of lymphocytes NFkB mRNA expression among control group due to absence of detectable lymphocytic infiltration.

Table (6) elucidate that grade (4) of tissue infiltrated PMN represent (66.7%) and the rest (33.3%) for grade (3) among CAG cases compared with (100%) for grade (1) of tissue infiltrated PMN among control group. low score of PMNs NFkB mRNA expression was detected in (66.66%) compared with (20%) for negative score and (13.34%) for Intermediate score of PMNs NFkB mRNA expression. PMNs NFkB mRNA expression was less than 5% in (13.33%) of cases with grade (3) compared with (6.66%) of cases with grade (4) PMNs NFkB mRNA expression. low score of PMNs NFkB mRNA expression was detected in (46.66%) of cases with grade (4) compared with (20%) of grade (3) PMNs infiltration. Regarding control group (100%) of cases the expression is less than 5% which considered negative score in the present study as shown in table (7) .

Table (8) elucidate that there was significant difference between PMNs and lymphocytes NFkB mRNA expression according to their sore of infiltration. High score of tissue NFkB mRNA expression was detected in 27 / 45 case (60%), while intermediate score was determined in 15 / 45 case (33.33%) .Low level of) tissue NFkB mRNA expression was detected in the rest 3 / 45 case (6.66%) as shown in table (9) and figure(2).

Regarding lymphocytes FAS mRNA expression this study elucidate that (73.34%) of cells express low level of Fas mRNA and the majority of them (40%) belong to grade (3) and the reminder (33.34%) of lymphocytes belong to grade (2).Intermediate score of expression was detected in (26.66%) of CAG cases that distributed equally with (13.33%) for grade (2) and grade (3) of lymphocytes infiltration .The total lymphocytes Fas mRNA expression was

detected in 24 / 45 CAG cases (53.34%) among grade (3) compared with 21(46.66%) /45 CAG cases among grade (2) as shown in table (10).

Table(11) shown that PMNs Fas mRNA expression was less than (5 %)which consider negative according to used score in (53.33%) of CAG cases in which 15 (33.34%)/45 belong to grade (3) and 9(20%)/45 case belong to grad(4)of PMNs infiltration compared with (40%) with low level of expression (33.34% for grade 4 and 6.67% for grade 3) on the other hand intermediate level of PMNs Fas mRNA expression was detected in (6.67%) of CAG cases that belong to grade (4) of PMNs infiltration. among control group in 100% of cases ,PMNs Fas mRNA expression was less than 5% which considered negative.

This study proved that there is a significant difference P<0.005 between PMNs and lymphocytes Fas mRNA expression as shown in table (12).Regarding Tissue Fas mRNA expression the present work proved that 24(53.34%)/ 45 of CAG cases characterized by Intermediate level of expression compared with 21(46.66%)/ 45 with high level of expression as shown in table (13) and figure (3).

This study explained that there was a statistical significant positive linear relationship between lymphocytes grade and PMN Fas mRNA expression (pearsons correlation coefficient (pn)= 0.326; p value =0.029), lymphocytes grade and Tissue Fas mRNA expression (pn)= 0.409; p value =0.005).

PMN grade has significant positive linear relationship with each of the following markers: Tissue Fas mRNA expression (pn)= 0.677; p value =0.000), Tissue NFkB mRNA expression (pn)= 0.326; p value =0.029), lymphocytes NFkB mRNA expression (pn)= 0.341; p value =0.022), PMN NFkB mRNA expression (pn)= 0.428; p value =0.003), Cag A mRNA expression (pn)= 0.500; p value =0.000).

lymphocytes Fas mRNA expression has significant positive linear relationship with PMN Fas mRNA expression (pn)= 0.510; p value =0.000) and Cag A mRNA expression (pn)= 0.500; p value =0.000).PMN Fas mRNA expression has significant positive linear relationship with Cag A mRNA expression (pn)= 0.473; p value =0.001) and negative linear relationship with tissue NFkB mRNA expression (pn)= -0.400; p value =0.007).

Tissue Fas mRNA expression has significant positive linear relationship with tissue NFkB mRNA expression (pn)= 0.420; p value =0.004); lymphocytes NFkB mRNA expression (pn)= 0.374; p value =0.011); PMN NFkB mRNA expression (pn)= 0.359; p value =0.016); Cag A mRNA expression (pn)= 0.540; p value =0.000),Cag A mRNA expression has significant positive linear relationship with lymphocytes NFkB mRNA expression (pn)= 0.459; p value =0.002); PMN NFkB mRNA expression (pn)= 0.690; p value =0.000).

Tissue NFKB mRNA expression has significant positive linear relationship with lymphocytes NFkB mRNA expression (pn)= 0.347; p value =0.020); PMN NFkB mRNA expression(pn)= 0.278; p value =0.064). lymphocytes NFkB mRNA expression has significant positive linear relationship with PMN NFkB mRNA expression (pn)= 0.461; p value =0.001).

Table (1) General description of age for patients that enrolled in the present study.

Age parameters	Patients (years)	Control group (years)
Minimum	18	23
Maximum	75	58
Mean	39.26	38
Std. Error	2.48	2.37
Std. Deviation	16.64	10.60
Range	57	35
Total number	45	20

Table (2): General description of Gender for patients that enrolled in the present study.

Gender	Frequency (%)	
	CAG cases	Control group
Male	33(73.3%)	10 (50%)
Female	12(26.7%)	10 (50%)
Total	45(100%)	20(100%)

Table (3): *H.pylori* Cag A and urease detection in CAG tissue samples and control group

H.pylori Cag A and urease detection	CAG cases	Control group
	No. (%)	No. (%)
H.pylori -ve Cag A mRNA expression/+ urease	30(66.7%)	0(0%)
H.pylori +ve Cag A mRNA expression/+ urease	15(33.3%)	0(0%)

Total	45(100%)	0(0%)

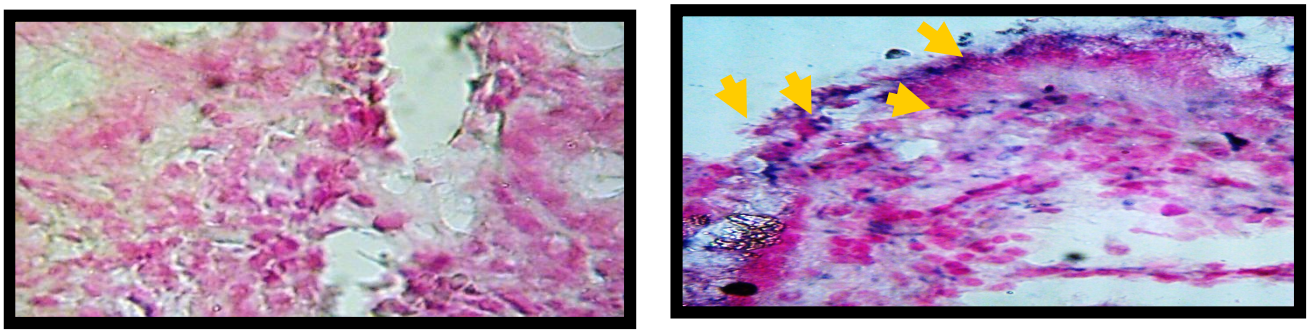


Figure (1) A :*In situ* hybridization for CagA Positive *H.pylori* *in* gastric tissue section .staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

B: *In situ* hybridization for CagA Positive *H.pylori* *in* gastric tissue section section for control group. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

Table (4): Grads of infiltrated grade in CAG cases and control group tissue lymphocytes

Tissue infiltrated	CAG cases	Control group
	Frequency (%)	Frequency (%)
Grade 0	0(0%)	20(100%)
Grade 1	0(0%)	0(0%)
Grade 2	21(46.7%)	0(0%)
Grade3	24(53.3%)	0(0%)
Total	45(100%)	20(100%)

Table (5) : Lymphocytes NFkB mRNA expression among (45) CAG cases

Marker	Score				Total no. (%)
Lymphocytes NFkB mRNA Expression	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	of expression
Grade 0	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 1	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 2	0(0%)	3(6.66%)	6(13.33%)	12(26.66%)	21(46.7%
Grade 3	0(0%)	3(6.66%)	9(20%)	12(26.66%)	24(53.3%
Total no. (%) of	0(0%)	6(13.34%)	15(33.34%)	24(53.33%)	45(100%)

Table (6): Grads of tissue infiltrated PMN grade in CAG cases and Control group

Tissue infiltrated PMN grade	CAG cases	Control group
	Frequency (%)	Frequency (%)
Grade 0	0(0%)	0(0%)
Grade 1	0(0%)	20(100%)
Grade 2	0(0%)	0(0%)

Grade 3	15(33.3%)	0(0%)
Grade4	30(66.7%)	0(0%)
Grade 5	0(0%)	0(0%)
Total	45(100%)	20(100%)

Table (7): PMNs NFkB mRNA expression among (45) CAG cases vs control group

Marker	Clinical presentation	Score				Total no. (%) of expression
PMNs NFkB mRNA expression		Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
Grade 0	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 1	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control	20(100%)	0(0%)	0(0%)	0(0%)	20(100%)
Grade 2	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 3	CAG	6(13.33%)	9(20%)	0(0%)	0(0%)	15(33.33%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade4	CAG	3(6.66%)	21(46.66%)	6(13.34%)	0(0%)	30(66.67%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 5	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total no. (%) of expression	CAG	9(20%)	30(66.66)	6(13.34%)	0(0%)	45(100%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	20(100%)

Table (8): Lymphocytes vs PMNs NFkB mRNA expression among (45) CAG cases

Marker	score				Total No. (%)	T-test P value
	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%		
Lymphocytes NFkB mRNA expression	0(0%)	6(13.33%)	15(33.33%)	24(53.33%)	45(100%)	P<0.005
PMNs NFkB mRNA expression	9(20%)	30(66.66%)	6(13.33%)	0(0%)	45(100%)	

Table (9): Tissue NFkB mRNA ex

Marker	score				Total No. (%)
	Negative	Low	Intermediate	High	
Tissue NFkB mRNA	0(0%)	3(6.66%)	15(33.33%)	27(60%)	45 (100%)

pression among (45) CAG case

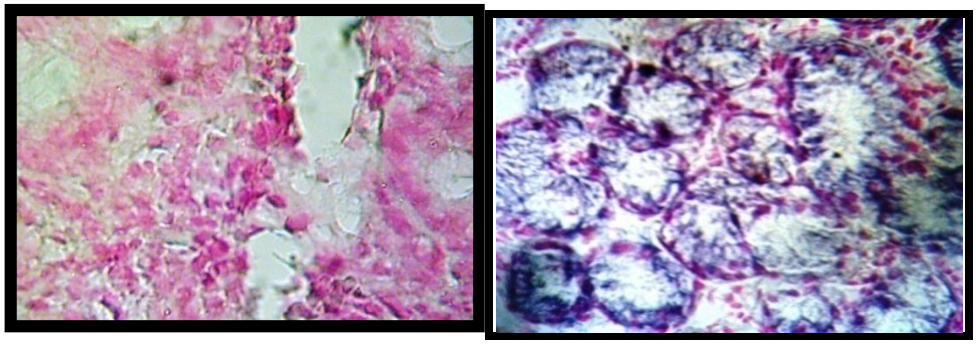


Figure (2):A : *In situ* hybridization for human NFkB in gastric tissue section. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

***B: In situ* hybridization for human NFkB in gastric tissue section for control group. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.**

Table (10): Lymphocytes Fas mRNA expression among (45) CAG cases

Marker			Score		Total no.(%)
Lymphocytes Fas mRNA expression	Negative	Low	Intermediate	High	
Grade 0	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 1	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 2	0(0%)	15(33.34%)	6(13.33%)	0(0%)	21(46.66%)
Grade 3	0(0%)	18(40%)	6(13.33%)	0(0%)	24(53.34%)
Total no.(%)	0(0%)	33(73.34%)	12 (26.66%)	0(0%)	45(100%)

Table (11): PMNs Fas mRNA expression among (45) CAG cases and control group

Marker	Clinical			Score		Total no.
PMNs Fas mRNA	presentation	Negative < 5%	Low 5-25%	Intermediate	High >50%	(%) of
Grade 0	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 1	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control group	20(100%)	0(0%)	0(0%)	0(0%)	20(100%)
Grade 2	CAG		0(0%)	0(0%)	0(0%)	0(0%)
	Control group	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 3	CAG	15(33.34%)	3(6.67%)	0(0%)	0(0%)	18(40%)
	Control group	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade4	CAG	9(20%)	15(33.34%)	3(6.67%)	0(0%)	27(60%)
	Control group	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade 5	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control group	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total no.	CAG	9(20%)	30(66.66)	6(13.34%)	0(0%)	45(100%)

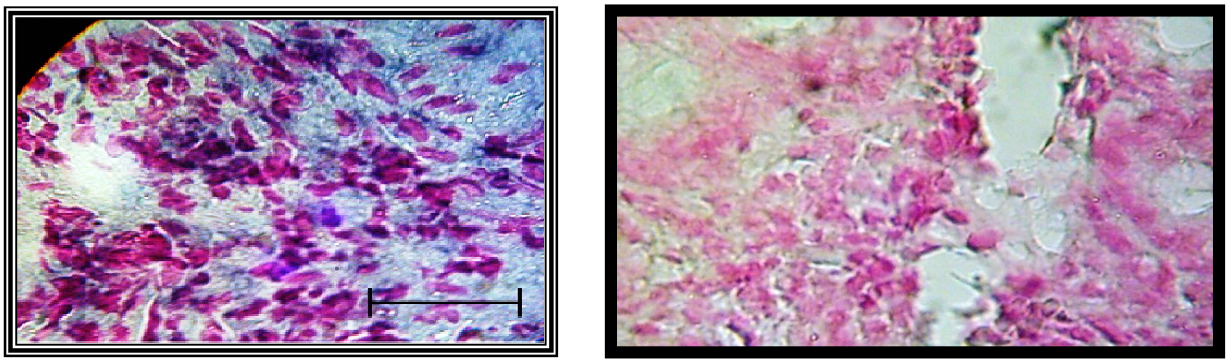
(%)	Control group	0(0%)	0(0%)	0(0%)	0(0%)	20(100%)

Table (12): Lymphocytes vs PMNs Fas mRNA Expression among (45) CAG cases

Marker	score				Total no.(%)	T-test P value
	Negative	Low	Intermediate	High		
Lymphocytes Fas	0(0%)	33(73.33%)	12(26.66%)	0(0%)	45(100%)	P<0.005
PMNs Fas mRNA	24(53.33 %)	18(40%)	3(6.66%)	0(0%)	45(100%)	

Table (13): Tissue Fas mRNA Expression among (45) CAG cases

Marker	score				Total no. (%) of
	Negative < 5%	Low 5-25%	Intermediate	High >50%	
Tissue Fas mRNA expression	0(0%)	0(0%)	24(53.34%)	21(46.66%)	45(100%)



Figure(3) :A : *In situ* hybridization for human Fas in gastric tissue section. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

B: In situ hybridization for human Fas in gastric tissue section for control group. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

Table (14): Correlations among different markers used in the present study

Parameter	pearsons correlation coefficient	PMN Fas mRNA expression	Tissue Fas mRNA expression	Cag A mRNA expression	Tissue NFkB mRNA	lymphocytes NFkB mRNA	PMN NFkB mRNA
lymphocytes grade	pn	0.326	0.409	0.094	-0.022	-0.098	-0.174
	p	0.029	0.005	0.537	0.885	0.521	0.253
PMN grade	pn	0.237	0.677	0.500	0.326	0.341	0.428

	p	0.117	0.000	0.000	0.029	0.022	0.003
lymphocytes	pn	0.510	0.116	0.511	0.162	0.016	0.159
Fas mRNA	p	0.000	0.447	0.000	0.288	0.916	0.296
PMN Fas	pn		0.247	0.473	-0.400	-0.085	0.065
mRNA	p		0.101	0.001	0.007	0.580	0.671
Tissue Fas	pn			0.540	0.420	0.374	0.359
mRNA	p			0.000	0.004	0.011	0.016
Cag A mRNA	pn				0.226	0.459	0.690
	p				0.136	0.002	0.000
Tissue NFKB	pn					0.347	0.278
mRNA	p					0.020	0.064
lymphocytes	pn						0.461
NFkB mRNA	p						0.001

Discussion

Atrophic gastritis is a histopathological entity characterized by chronic inflammation of the gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium and fibrous tissue. Atrophy of the gastric mucosa is the endpoint of chronic processes, such as chronic gastritis associated with *Helicobacter pylori* infection, other unidentified environmental factors, and autoimmunity directed against gastric glandular cells.

In this study the minimum age of individuals suffering from CAG was 18 years while maximum age was 75 years and the mean age was 39.26 years.

This result comes in agreement with (court *et al,* 2003) [17] how notice that chronic gastritis was 38% among the age group (25-35) years. While the prevalence among the age group more than 55 years represent 65 % .Frezza *et al,*(2001) [18] record that the incidence of CAG was higher among the age group over 60 years and they consider that old age was the most important risk factors for development of CAG.

During a comprehensive study, Zhang *et al.,* (2005)[19] elucidate that the distribution of chronic atrophic gastritis in less than 30 years to more than 70 years age groups was 33.3% to 43.5%, respectively. Zhang *et al.,* (2005) [19] explained that the distribution of *H.pylori* colonization at antrum was similar to corpus in patients with chronic gastritis before 50 years. These results suggest the distribution of *H.pylori* infection is pangastric in the younger patients. Salomaa-Ra¨sa¨nen *et al* (2004)[20] recorded that the incidence of CAG in association with H.pylori was 13.4% among the age group 15-49 years and reach to 41.5% in age more than 65 years .Progression of glandular atrophy seems to have a key role in the distribution of *H.pylori* colonization in this area. In younger there were not much glandular atrophy in the stomach, group which is beneficial for *H.pylori* colonization,

therefore *H.pylori* even colonized, however, colonization of the lumen of gastric glands by *H.pylori* eventually led to gastritis, glandular atrophy, and intestinal metaplasia, especially in antrum, but in the corpus it was very mild. Glandular atrophy in antrum may result in decreased acid output as a consequence of diminished gastrin release (Gillen *et al.,* 1998)[21] therefore *H.pylori* gradually decreased during the development of glandular atrophy in the antrum but not in corpus in the older age group.

In the present study males presented with CAG represent (*73.33%)* and the rest (*26.67%*) were females compared with (50%) for each gender in control group .In contrast, Frezza *et al*,(2001)[18] implicate that males represent(43.22%)of CAG cases where as (56.78%)among Italian patients attended for OGD were females. The Eurohepygast study group (2002) [22]were record high incidence of CAG among females (63%) compared with (37%) in males. The possible reason for such difference in results of the present local study and other previously mentioned world studies may be related to study design which was an epidemiological study and focus on the prevalence of *H.pylori* infection in relation with gender and the size of community under investigation that give a chance for difference in sample size of each gender type which is apparently larger in case of females in European communities .Emotional stress factor may have a role in susceptibility to *H.pylori* infection .Gender differences to *H.pylori* induced gastroduodenal diseases and in disease susceptibility may reflect differences in immune responses, hormonal effects and sex linked genetic factors and differences in bacterial colonization (Court *et al*.,2003) [17] .

H.pylori with negative Cag A mRNA expression *insitu* and positive production of urease was determined in 30(66.7%) Out of 45 patient, while in 15(33.3%) CAG cases *H.pylori* with positive Cag A mRNA expression *insitu* and positive production of urease was detected compared with negative results in all cases of control group .the results of the present study come in agreement with that of Figura *et al* ,(1998)[23] recorded that (44%) of cases presented with atrophic gastritis have *H.pylori* with positive Cag A mRNA expression *insitu* .They elucidate that a high frequency of Cag A + colonies was found in areas of gastric atrophy, whereas in areas of normal mucosa Cag A + colony frequency was similar to that found in areas of inflamed mucosa without atrophy. This finding may suggest that the pathogenic potential of Cag A + strains on the gastric mucosa may be exerted in full only when Cag A + colonies make up the majority of the organisms colonizing a particular gastric area. Hussein *et al* (2009)[24] recorded results very closely similar to that recorded in the present study, they found that (66.6%) of cases presented with chronic gastritis have *H.pylori* with positive Cag A. Furthermore, in Iran, histological evidence of mucosal atrophy was seen in 39% and 22% of the antral and corpus samples, respectively (Malekzadeh,2004)[25] In another study conducted in

Turkey, it was found that 43% of the *H. pylori*-infected subjects had atrophic gastritis(Fikret *et al*,2001)[26]

The present study revealed that heavy lymphocytes infiltration of tissue represent (53.3%) and (46.7%) for moderate lymphocytes infiltration. Regarding PMNs, grade represent (66.7%) and the rest (33.3%) for grade (3) among CAG cases.

This result come in agreement with that recorded by the Aspholm,(2004) [27]Who mentioned that *H. pylori* have adhesins proteins such as blood group antigen binding adhesin (BabA) which binds to fucosylated ABO blood group antigens on healthy gastric epithelial cells (i.e. Leb, A Leb, B Leb) , and the sialic acid binding adhesin (SabA) which binds to inflammation-associated sialylated glycans such as sialyl-Lewis x and sialyl-Lewis a (i.e. sLex and sLea) during the course of infection (Segal *et al.,*1999) [28] ; Ota *et al.*,(1998) [29].

Segal *et al.,(*1999) [28],mentioned that the adhesion process itself may not affect the structure or function of the host cell, microorganisms also produce toxins – enzymes that may ultimately damage host cells. *H. pylori* induces the morphological alterations in gastric epithelial cells . *H. pylori*-related chronic inflammation in gastric tissue has also been reported to modulate the glycosylation patterns of epithelial cells. Ota *et al.,*(1998) [29], have shown increased levels of sLea antigen in response to *H. pylori* infection and they have shown that sLex was upregulated in gastric epithelial cells due to *H. pylori* infection .In contrast, normal human gastric epithelial cells are essentially devoid of such sialylated glycoconjugates . In addition, recent studies have indicated that adherence of *H. pylori* induces cell proliferation and apoptosis during the early phase of chronic inflammation of the gastric mucosa (Ebert *et al.,* 2002) [30].

After adherence of *H. pylori* on the gastric epithelia, numerous cell associated bacterial proteins have been shown to play vital role in stimulation of the inflammatory response as soon as possible such as urease that protects the organism from acidic environment, lipopolysaccharide, outer membrane proteins (Mops), and bacterial flagellin.(Rachmilewitz *et al* ,2004)[31].

A characteristic feature of any bacterial infection is the migration of PMNs towards the site colonized by the infecting organism. Binding of *H. pylori* to the gastric epithelial cells induces the expression and secretion of IL-8 (McCormick *et al.,* 1998) [32]. In response to a chemotactic gradient from the site of infection, PMNs first adhere to,and then traverse, the vascular endothelium . This process involves the interaction between E-selectin, intercellular adhesion molecule (ICAM) 1 and 2 on the surface of the endothelium cell, and sLex and _2-integrins on the PMN surface (Slattery *et al.,* 2003) [33] . PMNs are rich in various sialylated glycoconjugates, which *H. pylori* can bind to. An

important consequence would be the bacterial interaction with, and activation of PMNs. Petersson *et al.* (in manuscript) [34] have demonstrated that SabA is essential for attachment and activation of human PMNs.

Mahdavi, (2004) [16] elucidate that attachment of *H.pylori* to gastric epithelial cells activates the type IV secretion system, which results in the injection of effector proteins into host cells. The effectors are thought to upregulate the activity of Nuclear Factor NF-kB, which, together with AP-I, leads to induction of IL-8 expression. This triggers transduction pathways and pedestal formation as a result of cytoskeletal rearrangements. Ismail *et al*, (2003) [36] mentioned that the leukocytes are programmed to reveal the healing process, but they can also enhance inflammation, which can proceed to tissue damage. The rolling and attaching phase in the capillaries is mediated by the selectin family, the E- and L-selectins, and their sialylated Lewis type of ligands. *H. pylori* adhesion stimulates the release of the IL-8 and induces expression of the intercellular adhesion molecule ICAM-1, which facilitates the migration of PMN cross the gastric epithelium.

Ismail *et al* ,(2003) [36) ; Olfat,(2003)[37] and Mahdavi,(2004) [16], proved that One of the primary host responses towards *H. pylori* infection is the recruitment of neutrophils and monocytes to the site of infection. This may attributed to bacterial proteins such as *H. pylori*'s neutrophil activating protein (HP-NAP), urease which is abundant extracellular proteins. HP-NAP is chemotactic for the human neutrophils and causes translocation of neutrophils from the blood stream to the infected stomach mucosa. In addition, HP-NAP binds to the neutrophils and thereby initiates the activation of these cells. HP-NAP was found to promote the adhesion of PMNs to endothelial cells by up regulating adhesion receptors of the 2-integrin family (Satin *et al.,* 2000) [38]. Satin and colleagues showed that HP-NAP stimulates (NADPH) oxidase assembly and production of reactive oxygen species (ROS). Also, as PMNs consistently outnumber macrophages in *H.pylori* infected rkholm *et al*., stomach, it induces a state of chronic acute inflammation. Previous reports of Bj (2000)[39] ; Amieva *et al*.,(2002) [40]; Kwok *et al*., (2002) [41] have suggested that *H. pylori* is capable of invading epithelial cells in the gastric mucosa. Dundon *et al.*,(2002) [42] and Shimoyama *et al .,*(2003) [43]elucidated that there is a good correlation between severity of mucosal damage and extent of neutrophils infiltration. As a consequence, it has been suggested that leukocytes may be responsible for the tissue damage seen during *H. pylori* infection.

For the first time in Iraq this study designed to demonstrate the expression of NFkB in inflammatory cells including lymphocytes and PMNs according to the grade of infiltration as well as gastric epithelial cells. There was significant difference between PMNs and lymphocytes NFkB mRNA expression according to their sore of infiltration. .

Unfortunately we have no any update information about similar study design to the present work, variety of literatures concentrating the pathological interaction of *H.pylori* with gastric tissue

Takenaka *et al*(2004) [44] ; Lee *et al* (2004) [45] and Lin *et al*(2005) [46] mentioned that the initial migration and activation of inflammatory cells into the gastric mucosa is dependent on the production of various proinflammatory cytokines such as IL-8, a potent neutrophil chemotactic and activating peptide produced by gastric epithelial cells. In addition to the *Cag* PAI and OipA, HSP60 and a polymorphism of *rpoB* gene, encoding the β-subunit of DNA-dependent RNA polymerase , are also *H. pylori* factors mediating IL-8 induction from epithelial cells.

Mandell *et al* (2004) [47]; Chang *et al* (2004) [48] and Torok (2005) [49] elucidated that the innate immune system is the first line of defense against invading pathogens. Toll-like receptors (TLRs) and Nod proteins are host molecules that recognize microbial components such as lipoproteins and peptidoglycan (TLR2),lipopolysaccharide (TLR4), flagellin (TLR5),CpG motifs of bacterial DNA (TLR9), and peptidoglycan (Nod1 and Nod2). Cytokine response to whole live *H. pylori* was mediated by TLR2, TLR5, and TLR9, but not by TLR4 .TLR2, TLR5, and TLR9 may thus be the dominant innate immune receptors for recognition of *H. pylori*.

Viala *et al*.(2004) [50] reported that *H. pylori* peptidoglycan was recognized by epithelial cells via Nod1 . The detection by this intracellular pathogen-recognition molecule of *H. pylori* peptidoglycan was dependent on its delivery to the host cells by the *Cag* PAI.

Chang *et al* (2004) [48] noticed that several novel signaling pathways mediated by *H. pylori* infection in gastric epithelial cells have also been investigated in the last year. NF-kB, NF-IL6, and cAMP response element (CRE) were found to be involved in COX-2 induction by *H. pylori* infection.

Cario *et al* (2000) [51] and Rosenstiel *et al* (2003) [52] proved that exposure to proinflammatory cytokines up- regulates membrane TLR and intracytoplasmic levels of NOD 2/CARD 15, making these activated cells more responsive to microbial signals. Ligation of these microbial recognition receptors has both physiologic and pathophysiologic consequences relevant to mucosal homeostasis and inflammation.

Stagg *et al*,(2003) [53], Rachmilewitz *et al*(2004) [54] and Cho *et al* (2007) [55] recorded that After sequential activation of NFkB several proinflammatory cytokines and adhesion molecules will be synthesized and produced via gastric epithelial cells such as IL-1, IL -2, IL-6, IL-12, IL-18, IL-23 and TNF. Chemokines synthesis will be increased such as IL-8; Groα, β; RANTES; MIP-2; IP-10. Adhesion molecules also increased in its expression such as ICAM-1, ELAM, VCAM, P-selectin. On the other hand Membrane receptors: IL-2R, CD95/APO-1(Fas receptor), Enzymes such as: COX-2, iNOS,

5-lipoxygenase, 12-lipoxygenase, SOD also increased in synthesis. Immunoregulatory molecules such as: MHC Class II and Costimulatory molecules such as CD40, CD80, and CD86 increased in synthesis.

This study elucidated that there was significant difference between PMNs and lymphocytes NFkB mRNA expression according to their site of infiltration this may be attributed to inhibitory signals that received from gastric epithelial cells producing molecules such as COX2 and iNOS that leads to downregulation of NFkB expression by its paracrine action on inflammatory cells and autocrine action on gastric epithelial cells.

This study proved that there is a significant difference P<0.005 between PMNs and lymphocytes Fas mRNA expression as .Regarding tissue Fas mRNA expression the present work proved that 24(53.34%)/ 45 of CAG cases characterized by Intermediate level of expression compared with 21(46.66%)/ 45 with high level of expression.

The results of the present work come in agreement with that recorded by Jones *et al* (2002)[56] ,they mentioned that both host determinants and bacterial factors are involved in mediating changes in gastric epithelial cell turnover. Peek *et al* (1997) [57]; Fan *et al* (1998) [58] and Jones *et al* (1999) [59] have demonstrated that infection with CagA+ *H. pylori* strains is associated with enhanced proliferation without a corresponding increase in apoptosis. Wagner *et al* (1997) [60] mentioned that In vitro studies indicate that the bacterium is capable of directly inducing apoptosis of gastric cells . Fan and colleagues (1998) [58] provided evidence that binding of urease to class II major histocompatibility complex molecules mediates apoptosis in vitro. In addition, *H. pylori* infection increases the sensitivity of gastric epithelial cells to Fas-triggered apoptosis by enhancing Fas receptor expression . Houghton *et al* (1999) [61] mentioned that Inflammatory cytokines present during H. pylori infection, such as IFN-γ also enhance activation of the Fas signaling pathway in vitro.

Jones *et al* (1997) [62] mentioned that among *H. pylori*-infected children, gastric epithelial cell apoptosis returns to baseline levels only following both eradication of bacterial colonization and resolution of the accompanying inflammatory cell infiltrate . These findings suggest that immune-mediated cell death through the Fas pathway could contribute to the apoptosis that is observed during infection in vivo.

Fas-mediated gastric mucosal apoptosis is gaining attention as a cause of tissue damage due to *H. pylori* infection. Houghton *et al* (2000) [61] elucidate that *H. pylori* have direct effects on Fas-mediated apoptosis in a nontransformed gastric mucosal cell line (RGM-1), also they demonstrate the effects of the inflammatory environment established subsequent to *H. pylori* infection, on Fas-

mediated apoptosis in the same cell line. Exposure to *H. pylori*-activated peripheral blood mononuclear cells (PBMCs), but not *H.pylori* itself, induced Fas antigen (Fas Ag) expression, indicating a Fas-regulatory role for inflammatory cytokines in this system. Of various inflammatory cytokines tested, only IL 1β and TNFα induced Fas Ag expression, and removal of either of these from the conditioned medium abrogated the response. When exposed to Fas ligand, RGM-1 cells treated with PBMC-conditioned medium underwent massive and rapid cell death, interestingly, with a minimal effect on total cell numbers early on. Cell cycle analysis revealed a substantial increase in S phase cells among cells exposed to Fas ligand, suggesting an increase in their proliferative response. Taken together, these data indicate that the immune environment secondary to *H. pylori* infection plays a critical role in priming gastric mucosal cells to undergo apoptosis or to proliferate based upon their Fas Ag status.

Jones *et al* (1999)[59] and Wagner *et al*(1997) [60] mentioned that *H. pylori* infection is associated with elevated levels of both mucosal apoptosis and proliferation .The initiation and the regulation of the pathways that promote these paradoxical cellular responses are still unclear. Houghton *et al* (1999) [61] recorded that in *H.pylori*-infected human biopsy specimens, there was concomitant Fas antigen (Fas Ag) expression and gastric mucosal apoptosis, suggesting a role for Fas signaling in H.pylori-associated apoptosis . Fas Ag is a transmembrane receptor, which when bound specifically to its ligand (Fas L) trimerizes and initiates a cascade of events resulting in apoptosis in a variety of cell settings (Giordano *et al*,(1997) [63] and Katsikis *et al*,(1995) [64] .

Katsikis *et al*,(1995) [64] and Freiberg *et al* (1997) [65] recorded that the pathway can be modulated at various points throughout, including regulation of the number of Fas receptors on the cell membrane as well as regulation of the availability of FasL . Houghton *et al* (1999) [61] Fas Ag and Fas L expression have been shown to be regulated at the mRNA level in various cell types by inflammatory cytokines such as IL-1β, IL-2, TNF-α, and IFN-γ . Although the Fas pathway has been well characterized in the immune system, less is known about the role this pathway plays in nonlymphoid tissue.

D'Elios *et al* ,(1996) [66] and Harris *et al* (1996) [67] found that H. pylori infection is associated with increased mucosal inflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α and IFN-γ .they found that Production of IL-1 β, IL-6, IL-8,and TNF- α has been demonstrated in H. pylori-stimulated peripheral blood mononuclear cell (PBMC) cultures, suggesting that immune cells may be the source of the mucosal cytokines found clinically. Since cytokines have shown the capacity to induce Fas Ag expression in malignant gastric cell lines postulated that the cytokines generated during the immune response to H. pylori could prime nonmalignant gastric tissue for apoptosis by

increasing mucosal expression of Fas Ag. In this scenario, Fas L, which is expressed on lymphocytes present in infected gastric tissue could trigger Fas mediated apoptosis.

The differences in the expression of Fas gene between lymphocytes and PMNs and between infiltrated cells and gastric epithelial cells may be associated with variability in response to inducible stimuli in the form of micro environmental cytokines profile that act in autocrine or paracrine activity or both .Harris *et al* (1996) [67] and Maekawa *et al* ,(1997) [68].

Here we have possible scenario of expression of gastric tissue Fas mRNA, NFkB mRNA expression as well as lymphocytes NFkB mRNA expression, PMN NFkB mRNA expression , PMN Fas mRNA, lymphocytes Fas mRNA , PMN grade, lymphocytes grade, Cag A mRNA expression

After entrance of H*. pylori* to the gastric environment, *H. pylori* use adhesins proteins such as (BabA) which binds to fucosylated ABO blood group antigens on healthy gastric epithelial cells (i.e. Leb, A Leb, B Leb) , and (SabA) which binds to inflammation-associated sialylated glycans such as (sLex and sLea) during the course of infection (Segal *et al.,*1999)[28] , Microorganisms also produce toxins – enzymes that may ultimately damage host cells. *H. pylori* induces the morphological alterations in gastric epithelial cells. After adherence of *H. pylori* on the gastric epithelial cells numerous cell associated bacterial proteins have been shown to play vital role in stimulation of the inflammatory response such as urease that protects the organism from acidic environment converting urea to ammonia and carbon dioxide also urease that distributed after secretion on the bacterial cell surface bind to MHC class II that express on gastric epithelial cells making attachment with gastric epithelial cells firm enough to establish a pathological effects on host cells . *H. pylori* use lipopolysaccharide, outer membrane proteins (Mops), and bacterial flagellin for attachment with specific receptor such as MUC5 that express on gastric epithelial cells.

Attachment of H.pylori to gastric epithelial cells activates the type IV secretion system, which results in the injection of effector proteins such as Cag toxin into host cells. Cag toxin are thought to upregulate the activity of Nuclear Factor NF-κB, which, together with AP-I, leads to induction of IL-8 expression. This triggers transduction pathways and pedestal formation as a result of cytoskeletal rearrangements. The leukocytes are programmed to reveal the healing process, but they can also enhance inflammation, which can proceed to tissue damage. The rolling and attaching phase in the capillaries is mediated by the selectin family, the E- and L-selectins, and their sialylated Lewis type of ligands. H. pylori adhesion stimulates the release of the IL-8 and induces expression of the intercellular adhesion molecule ICAM-1, which facilitates the migration of PMNs and lymphocytes across the gastric epithelium.

Exposure of gastric epithelial cells to proinflammatory cytokines produced from PMNs and lymphocytes up- regulates membrane TLR and intracytoplasmic NOD 2/CARD 15 levels, making these activated cells more responsive to microbial signals. Ligation of these microbial recognition receptors has both physiologic and pathophysiologic consequences relevant to mucosal homeostasis and inflammation. After activation of NFkB several proinflammatory cytokines and adhesion molecules will be synthesized and produced via gastric epithelial cells such as IL-1, IL -2, IL-6, IL-12, IL-18, IL-23 and TNF. Al so chemokines synthesis will be increased such as IL-8; Groα, β; RANTES; MIP-2; IP-10. Adhesion molecules also increased in its expression such as ICAM-1, ELAM, VCAM, P-selectin. On the other hand Membrane receptors: IL-2R, CD95/APO-1(Fas receptor), Enzymes such as: COX-2, iNOS, 5-lipoxygenase, 12-lipoxygenase, SOD also increased in synthesis. Immunoregulatory molecules such as: MHC Class II and Costimulatory molecules such as CD40, CD80, and CD86 increased in synthesis. All these events lead to increase in expression of Fas receptor on the PMNs and PMNs NFKB in association with lymphocytes grade and the reverse is true in case of lymphocytes Fas receptor and lymphocyte NFKB as well as gastric epithelial cell Fas receptor and NFKB which is affected by H.pylori Cag A and the chemokines in gastric microenvironment.

In conclusion we found that NFkB play vital role in up regulation of expression Fas receptor on gastric epithelial cells and PMNs and lymphocytes in *H.pylori* $^{cagA+/urease+}$ associated chronic gastritis which trigger Fas mediated apoptosis in gastric epithelial cells mainly and PMNs and lymphocytes which finally leads to typical histopathological features of chronic atrophic gastritis.

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