ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

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

Ali Ibrahim Ali Al-Ezzy
Department of pathology, College of Veterinary medicine –Diyala University –Diyala-Iraq.

Manuscript Info

Manuscript History:

Received: 25 November 2013
Final Accepted: 22 December 2013
Published Online: January 2014

Key words:
atrophic gastritis, NFkB, Fas, *H.pylori* CagA+/urease+,Insitu hybridization

Corresponding Author

Assist.Prof. Ali Ibrahim Ali
Email:
aliibrahim_75@yahoo.co.uk
alazziibrahim @gmail.com

Abstract

Chronic atrophic gastritis (CAG) characterized by chronic inflammation of gastric mucosa with loss of gastric glandular cells and replacement by intestinal-type epithelium and fibrous tissue. CAG may be associated with *Helicobacter pylori* infection. This study was designed to determine the role of NFkB signaling on Fas receptor mediated apoptosis in *H.pylori* Cag A^+ associated CAG. According to exclusion criteria (45) CAG patients and (20) control were chosen. Multiple gastric mucosal biopsies were taken for rapid urease test and preparation of slides from paraffin blocks. Slides stained with haematoxylin-eosin for histopathology and grading of PMNs and lymphocytes. Biotinylated DNA probe for human NF-KB and FAS genes were used for detection of their expression in tissue lymphocytes, PMNs and gastric epithelial cells using ISH technique. Biotinylated DNA probe for *H.pylori* Cag A gene was used for detection of its expression in gastric epithelial cells. This study revealed that lymphocytes grade has positive correlation with Fas mRNA expression in PMN and gastric epithelial cells. PMN grade has positive correlation with tissue Fas mRNA; tissue NFkB mRNA; Cag A mRNA; lymphocytes and PMN NFkB mRNA expression. Lymphocytes Fas mRNA has positive correlation with PMN Fas mRNA and Cag A mRNA expression .Tissue Fas mRNA has positive correlation with tissue NFkB mRNA; Cag A mRNA ,lymphocytes and PMN NFkB mRNA expression. Tissue NFKB mRNA has positive correlation with lymphocytes and PMN NFkB mRNA expression. This study concludes that NFkB play vital role in Immunopathology of *H.pylori* Cag A^+ associated gastritis that leads finally to atrophic changes.

1. Introduction

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. CAG may be associated with *Helicobacter pylori* infection as well as unidentified environmental factors and autoimmunity directed against gastric glandular cells **(1)** .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**(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) **(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 **(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 **(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 .it was demonstrated that *H. pylori* could activate NF-KB via a signaling pathway involving IKK and NIK **(1).** Given that many cell types become more sensitive to TNF-α or *H. pylori* induced apoptosis after the suppression of NF-KB activity **(8),** 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 **(9**). These conflicting observations indicate that the role of NF-KB in *H.pylori*-induced apoptosis in gastric epithelial cells has not been clarified.
This study designedto determine the role of NFkB signaling on Fas receptor mediated apoptosis in *H.pylori* Cag A^+ associated CAG.

2. Materials and methods

2.1. Patients

The subjects of the present study were selected from Patients attending the gastroenterology unit of digestive and hepatic diseases teaching hospital in Baghdad. The present research was approved by ethics committee of Diyala University, College of Medicine. At first the aim of study was explained for all participants and after obtaining their oral consent they have been studied. The subjects were diagnosed as having atrophic gastritis by upper gastrointestinal endoscopy from May 2009 to December 2010.Forty five patients suffering from chronic atrophic gastritis were chosen. Twenty apparently healthy volunteer were enrolled as control.

2.2. Exclusion criteria used in the study

Exclusion criteria were as follows:

a) Patients who had undergone or were currently undergoing *H.pylori* eradication therapy.
b) Patients who had received antiulcer treatment in the last three months , and still receiving proton-pump inhibitors (PPI) or H 2 receptor blockers
c) Patients who had received nonsteroidal anti-inflammatory drugs (NSAIDs) in the two months before the examination
d) The result of rapid urease test was negative

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.

2.3. 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. Rest biopsy specimens were fixed with 10% buffered formalin for preparation of paraffin embedded tissue blocks to prepare slides for histological examination using haematoxylin-eosin and Giemsa stains. Biopsy specimens obtained from the antrum were used to evaluate the grades of Polymorphonuclear leukocytes and lymphocytes infiltration according. The presence of *H.pylori* in tissue section was established by typical appearance on scanning along the mucosal surface and the individual gastric pits.

2.4. DNA Probes and DNA Probe hybridization/Detection System :

a)Biotinylated long DNA probe for human Fas Gene, Cat. No.: IH-60047(fas-6001-B). (Maxim biotech-USA);b) Biotinylated long DNA probe for human NF-KB gene, Cat. No. IH-60031) (Maxim biotech-USA).*c)* Biotinylated long DNA probe for H.pylori/ Cag A Gene, Cat. No.: IH-60061(HPY-6001-B) (Maxim biotech-USA).d) The DNA Probe hybridization/Detection System – In Situ Kit (Maxim biotech-USA).

2.5. Biopsy urease test (BUT):

The biopsies were placed directly into the rapid urea medium at the time of endoscopy**.** Positive results indicated by development of a pink-red or red-violet color **(10)**.

2.6. Insitu hybridization procedure

Human (NF-KB and Fas) genes as well as Cag A gene expression for*H.pylori* were detected in 4-6 μm thickness serial gastric mucosal tissue sections fixed on positively charged slides. According to Maxim biotech instruction manual **(11)**. The examination and scoring were done under light microscope by a pathologist at powerX400 according to the scoring system shown in table I.

Table (I) Scoring system used in Insitu hybridization (12), (13)

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

2.7. Scoring system for PMNs and lymphocytes(14)

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 PMN /hpf**
Grade 5	**pit abscess**

3. 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.

4. Results:

The demographic characteristics of patients and control are displayed in table (1). Insitu *H.pylori* expression of Cag A mRNA and production of urease are displayed in table (2) and figure (1). Table (3) 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. Score of lymphocytes NFkB mRNA expression according to tissue infiltrated lymphocytes grade was explained in table (4). Table (5) 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. Score of PMNs NFkB mRNA expression according to tissue infiltrated PMNs grade was explained in table (6).

There was significant difference between PMNs and lymphocytes NFkB mRNA expression according to their sore of infiltration as shown in table (7). 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 (8) and figure(2). Lymphocytes FAS mRNA ex-

pression according to grade of lymphocytic infiltration is elucidated in table (9).PMNs Fas mRNA expression among (45) CAG cases and control group according to PMNs grade are explained in table (10).
This study proved that there is a significant difference P<0.005 between PMNs and lymphocytes Fas mRNA expression as shown in table (11). Intermediate level of expression Fas mRNA detected in 24(53.34%)/ 45 of CAG cases compared with 21(46.66%)/ 45 with high level of expression as shown in table (12) and figure (3).Correlations among different markers used in the present study are explained in table (13).

Table (1): demographic Parameters for patients that enrolled in the present study.

Parameters		Patients	Control group
Age(years)	Minimum(years)	18	23
	Maximum(years)	75	58
	Mean± Std. Error	39.26±2.48	38±2.37
	Range	57	35
Gender	Male	33(73.3%)	10 (50%)
	Female	12(26.7%)	10 (50%)
Total number		45	20

Table (2): *H.pylori* Cag A and urease detection in gastric mucosal biopsies of CAG 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%)

Table (3): Grads of tissue infiltrated lymphocytes in CAG cases and control group

Tissue infiltrated lymphocytes Grade	CAG cases	Control group
	No. (%)	No. (%)
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 (4): Score of Lymphocytes NFkB mRNA expression among (45) CAG cases according to tissue infiltrated lymphocytes Grade

Marker	Score of Lymphocytes NFkB mRNA Expression				Total No. (%) of expression
Tissue infiltrated lymphocytes Grade	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
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 Expression	0(0%)	6(13.34%)	15(33.34%)	24(53.33%)	45(100%)

Table (5): Grads of tissue infiltrated PMNs in CAG cases and Control group

Tissue infiltrated PMNs grade	CAG cases No. (%)	Control group No. (%)
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 (6): PMNs NFkB mRNA expression in (45) CAG cases vs control group according to PMNs grade

Marker	Clinical presentation	Score of PMNs NFkB mRNA expression				Total No. (%) of expression
Grads of tissue infiltrated PMNs		Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
Grade 0	CAG	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	Control group	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%)	0(0%)
	Control group	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 group	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 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. (%) of expression	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 (7): score of 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 (8): score of tissue NFkB mRNA expression among (45) CAG case

Marker	Score				Total No. (%)
	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
Tissue NFkB mRNA expression	0(0%)	3(6.66%)	15(33.33%)	27(60%)	45 (100%)

Table (9): Lymphocytes Fas mRNA expression among (45) CAG cases according to tissue infiltrated Lymphocytes grade

Marker	Score of Lymphocytes Fas mRNA expression				Total No.(%)
Tissue infiltrated Lymphocytes grade	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
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 (10): PMNs Fas mRNA expression among (45) CAG cases and control group according totissue infiltrated PMNs grade

Marker	Clinical presentation	Score of PMNs Fas mRNA Expression				Total No. (%) of expression
PMNs grade		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 group	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	12(26.67%)	3(6.67%)	0(0%)	0(0%)	15(33.34%)
	Control	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Grade4	CAG	9(20%)	17(37.78%	4(8.88%)	0(0%)	30(66.66%)
	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 group	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total no. (%) of expression	CAG	21(46.67%)	20 (44.44%)	4(8.88%)	0(0%)	45(100%)
	Control group	20(100%)	0(0%)	0(0%)	0(0%)	20(100%)

Table (11): Lymphocytes vs PMNs Fas 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 Fas mRNA expression	0(0%)	33(73.33%)	12(26.66%)	0(0%)	45(100%)	P<0.005
PMNs Fas mRNA expression	21(26.66%)	20(44.44%)	4(8.88%)	0(0%)	45(100%)	

Table (12): Tissue Fas mRNA Expression among (45) CAG cases

Marker	Score				Total No. (%) of expression
	Negative < 5%	Low 5-25%	Intermediate 26-50%	High >50%	
Tissue Fas mRNAexpression	0(0%)	0(0%)	24(53.34%)	21(46.66%)	45(100%)

Table (13): Correlations among different markers used in the present study

Parameter	Pearson's correlation coefficient	PMN Fas mRNA expression	Tissue Fas mRNA expression	Cag A mRNA expression	Tissue NFkB mRNA	lymphocytes NFkB mRNA	PMN NFkB mRNA expression
lymphocytes grade	pn	0.326	0.409	0.094	-0.022	-0.098	-0.174
	P. value	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. value	0.117	0.000	0.000	0.029	0.022	0.003
lymphocytes Fas mRNA expression	pn	0.510	0.116	0.511	0.162	0.016	0.159
	P. value	0.000	0.447	0.000	0.288	0.916	0.296
PMN Fas mRNA Expression	pn		0.247	0.473	-0.400	-0.085	0.065
	P. value		0.101	0.001	0.007	0.580	0.671
Tissue Fas mRNA Expression	pn			0.540	0.420	0.374	0.359
	P. value			0.000	0.004	0.011	0.016
Cag A mRNA Expression	pn				0.226	0.459	0.690
	P. value				0.136	0.002	0.000
Tissue NFKB mRNA	pn					0.347	0.278
	P. value					0.020	0.064
lymphocytes NFkB mRNA expression	pn						0.461
	P. value						0.001

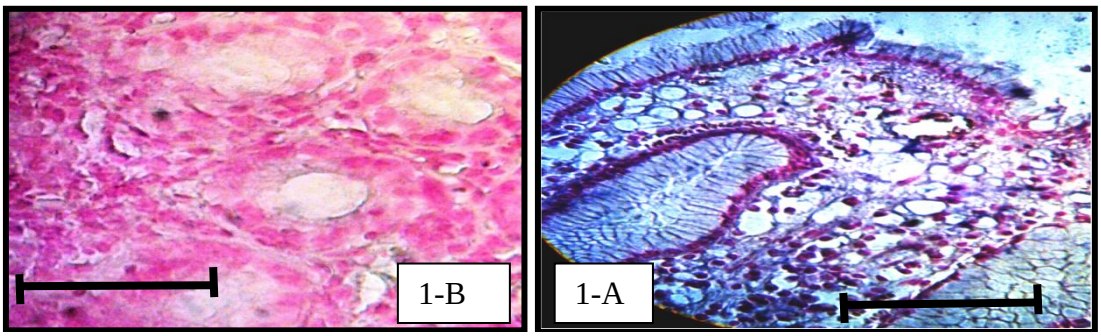

Figure (1) In situ hybridization using biotinylated Cag A DNA probe for detection of CagA expression

A) Positive *H.pylori* CagA expression in gastric tissue section .staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

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

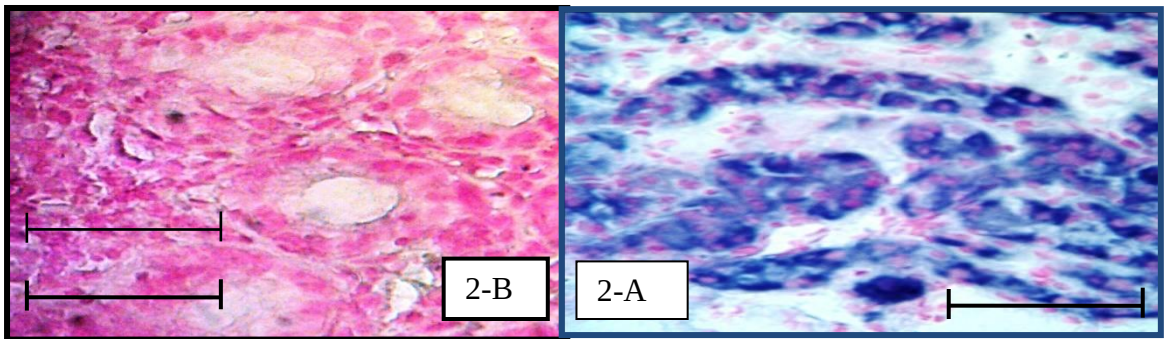

Figure (2): In situ hybridization for human NFkB in gastric tissue section using biotinylated DNA probe.
A)Positive In situ hybridization for human NFkB in gastric tissue section. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.
B)Negative in situ hybridization for human NFkB in gastric tissue section for control group. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

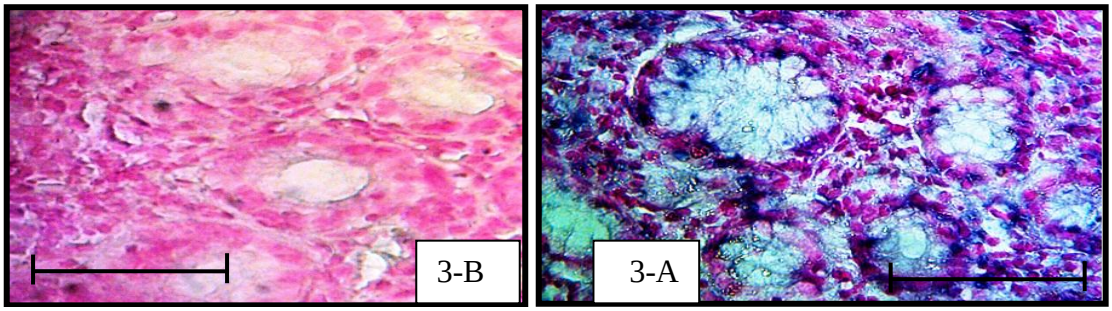

Figure (3):In situ hybridization for human Fas in gastric tissue section using biotinylated DNA probe.
A) In situ hybridization for human Fas in gastric tissue section. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.
B) Negativein situ hybridization for human Fas in gastric tissue section for control group. Staining by BCIP/NBT (bluish purple) counterstained with nuclear fast red. Bar size=50µm.

5. Discussion:

In this study the range of age in *H.pylori* associated CAG patients was (18 – 75) years. This come in concordance with records that the incidence of *H.pylori* associated CAG was higher among the age group over 60 years in comparison with younger age group. Old age was the most important risk factors for development of CAG **(15), *(16)*, (17), (18).**In younger age group there were not much glandular atrophy in the stomach, which is beneficial for *H.pylori* colonization in gastric gland lumen that eventually led to gastritis, glandular atrophy, and intestinal metaplasia, especially in antrum. Glandular atrophy in antrum may result in decreased acid output as a consequence of diminished gastrin release therefore *H.pylori* gradually decreased during the development of glandular atrophy in the antrum but not in corpus in the older age group.

The possible effect of gender in susceptibility to CAG was controversial **(15), *(16)*, (19)**.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.Gender differences in *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 also Emotional stress factor may have a role in susceptibility to *H.pylori* infection ***(16)***. Colonization of *H.pylori*cag A+/ urease + in atrophic gastric tissue was determined in (33.3%) of cases while in (66.7%) cases *H.pylori* cag A-/ urease + was detected compared with negative results in all cases of control group .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**(20) ,(21) ,(22) ,(23).**

The possible explanation for heavy lymphocytes and moderate to heavy PMNs infiltration that detected in the present study may attributed to the fact that *H.pylori* have adhesins proteins (BabA) which binds to fucosylated ABO blood group antigens (Leb, A Leb, B Leb)on healthy gastric epithelial cells during colonization , 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) that increased in its expression during the course of infection **(14),(24) ,(25) (26)** .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 such as *Cag* PAI and OipA, HSP60 as well as a polymorphism of *rpoB* gene, encoding the β-subunit of DNA-dependent RNA polymerase. These effectors are upregulate the activity of Nuclear Factor NF-kB, which, together with AP-I, leads to induction of IL-8 expression (27) , (28) , (29) . In response to a chemotactic gradient of IL-8 from the site of infection and activating peptide produced by gastric epithelial cells as well as H. pylori -neutrophils activating protein (HP-NAP) and urease which is abundant extracellular proteins , PMNs first adhere to, and then traverse, the vascular endothelium . This process involves the interaction between E-selectin, ICAM 1 and 2 on the surface of the endothelium cell, and sialylated glycans (sLex) and type 2-integrins on the PMN surface . Binding to and activation of human PMNs with H. pylori occurs using sialylated glycoconjugates on PMNs, and H. pylori SabA and HP-NAP **(30) , (31),(32),(33) ; (34) .** HP-NAP stimulates (NADPH) oxidase assembly and production of reactive oxygen species (ROS). Heavy PMNs infiltration in *H.pylori* infected stomach induces a state of chronic acute inflammation. The atrophic changes that detected in subjects of this study indicate the 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. Heavy lymphocytic infiltration supports the suggestion that H. pylori is capable of invading epithelial cells in the gastric mucosa **(35) ,(36),(37) , (38) , (39) .**

The difference in the grade of lymphocytes and PMNs infiltration maybe attributed to presence of different host molecule that recognize *H.pylori* components through which the different cytokines response was mediated. The extracellular recognition receptors such as Toll-like receptor 2(TLRs) recognize lipoproteins and ***H. pylori*** peptidoglycan, lipopolysaccharide (TLR4), flagellin (TLR5),CpG motifs of bacterial DNA (TLR9).intracellular recognition receptors (Nod1 and Nod2), recognize *H.pylori* peptidoglycan and lipoproteins upon its delivery to gastric epithelial cells by the *Cag* PAI (40), (41) (42) (43). As a results of recognition ,several novel signaling pathways mediated by *H. pylori* infection in gastric epithelial cells including NF-kB signaling pathway .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. Immunoregulatory molecules such as: MHC Class II on gastric epithelial cells and PMNs and lymphocytes as well as Costimulatory molecules such as CD40, CD80, and CD86 increased in synthesis.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.

Proinflammatory cytokines in microenvironment up-regulates membrane TLR and NOD 2/CARD 15, making activated gastric epithelial cells more responsive to H. pylorisignals and infiltration of PMNs and lymphocytes started**. (44), (45) , (46) .** if subject under investigation had receive anti-inflammatory drugs for adequate time before endoscopy the level of proinflammatory cytokines will be decreased and the *H.pylori* recognition receptor such as TLR and intracytoplasmic levels of NOD 2 will be decreased and the level of lymphocytes and PMNs infiltration will be reduced**(47), (48)** and finally the PMNs and lymphocytes NFkB mRNA expression fluctuated from high to intermediate level also this difference in NFkB mRNA expression between PMNs and lymphocytes in score of NFkB mRNA expression according to their grade of infiltration may be attributed to inhibitory signals that received from gastric epithelial cells producing molecules such as COX2 and iNOS that leads to down regulation of NFkB expression by its paracrine action on inflammatory cells and autocrine action on gastric epithelial cells.

One of possible explanation of increased level of gastric Fas expression may be attributed direct effect of H. pylori on gastric epithelial cells due to binding of H. pylori urease to gastric epithelial class II MHC molecules which leads to the level of gastric epithelial cells Fas expression and increased tendency to undergo Fas mediates apoptosis. **(49).**this explanation supported by observation that gastric epithelial cell apoptosis returns to baseline levels only following both eradication of H. pylori colonization and resolution of the accompanying inflammatory cell infiltrate which suggest that immune-mediated cell death through the Fas pathway could contribute to the apoptosis that is observed during infection in vivo **(50).** The positive feedback in the level of Fas expression between gastric tissue and PMNs and Lymphocytes supported by the effect of CagA injected directly through type IV secretion system upon colonization as direct inducer beside Inflammatory cytokines presents during H. pylori infection, such as IFN-γ which enhance activation of the gastric epithelial as well as inflammatory cells Fas signaling pathway **(51)**. an indirect pathway for Fas expression may be via Binding of *H.pylori* to infiltrated lymphocytes and PMNs directly using sialylated glycoconjugates on PMNs, and H. pylori SabA and HP-NAP that enhance Fas expression on inflammatory cells in microenvironment and the activated cells produce cytokines such as IFN-γ ,IL 1β , IL-2 and TNFα which enhance activation of the gastric epithelial Fas

signaling pathway or using alternative indirect way , *H. pylori*-activated PMNs can directly activates the gastric epithelia cells to express Fas antigen, which when bound specifically to its ligand (Fas L) expressed on lymphocytes and PMNs present in infected gastric tissue, trimerizes and initiates a cascade of events resulting in apoptosis **(52),(53).**

The differences in the score of expression of Fas gene between lymphocytes and PMNs and between infiltrated cells and gastric epithelial cells may be associated with variability in immune cells cytokines profile such as IL-1β, IL-6, IL-8, and TNF-α and IFN-γ in response to inducible H. pylori stimuli that act in autocrine or paracrine activity or both(54),(55) (56).

6. Conclusion:

In conclusion,NFkB play vital role in Immunopathology of *H.pylori* Cag A^+ associated gastritis that leads finally to atrophic changes.

References:

1. Maeda S, Yoshida H, Ogura K, Mitsuno Y, Hirata Y, Yamaji Y, Akanuma M, Shiratori Y, and Omata M.(2000):H. pylori activates NF-B through a signaling pathway involving IB kinases, NF-B-inducing kinase, TRAF2, and TRAF6 in gastric cancer cells. Gastroenterology.119: 97–108.
2. Naumann M. Nuclear factor-B activation and innate immune response in microbial pathogen infection. Biochem-Pharmacol(2000), 60: 1109–1114.
3. Kim JM, Kim JS, Jung HC, Song IS, and Kim CY. (2000):Upregulated cyclooxygenase-2 inhibits apoptosis of human gastric epithelial cells infected with Helicobacter pylori. Dig Dis Sci.,45: 2436–2443.
4. Kim JM, Kim JS, Jung HC, Song IS, and Kim CY. (2002):Upregulation of inducible nitric oxide synthase and nitric oxide in Helicobacter pylori-infected human gastric epithelial cells: possible role of interferon-in polarized nitric oxide secretion. Helicobacter ,7: 116–128,.
5. Jobin C and Sartor RB. (2000): The IB/NF-B system: a key determinant of mucosal inflammation and protection. Am J Physiol Cell Physiol,278: C451–C462,.
6. MacDonald TT and Pettersson S. (2000):Bacterial regulation of intestinal immune responses. Inflamm Bowel Dis ,6: 116–122.
7. Elewaut D, DiDonato JA, Kim JM, Troung F, Eckmann L, and Kagnoff MF. (1999): Nuclear factor-B is a central regulator of the intestinal epithelial cell innate immune response induced by infection with enteroinvasive bacteria. J Immunol .,163: 1457– 1466.
8. Schmid RM and Adler G. (2000):NF-B/Rel/IB: implications in gastrointestinal diseases. Gastroenterology .118: 1208–1228,
9. Lim JW, Kim H, and Kim KH. (2001): NF-B, inducible nitric oxide synthase and apoptosis by Helicobacter pylori infection. Free RadicBiol Med,31: 355–366,
10. Forbes, B.A., *et al.* (1998):Bailey and Scott's Diagnostic Microbiology, 10th ed. C.V. , Mosby Company, St. Louis, MO.
11. *Insitu* hybridization detection system available from http:// www.maximbiotech.com
12. AL-Ezzy, Ali I. (2006): Molecular and Immunopathological Role of FAS-FASL Apoptotic pathway in *H.pylori* CagA positive associated Gastroduodenal ulcer versus Chronic atrophic Gastritis In Iraqi patients. PhD thesis , ,College of medicine ,Al-Nahrain university, Iraq
13. Kadhim HS. (2004): possible role of cell cycle regulatory proteins and NFKB on the pathogenesis of transitional cell carcinoma of the bladder.PhD thesis .College of medicine .Al-Nahrain university.
14. Mahdavi.J. (2004): *Helicobacter pylori* adhesion and pathoadaptation :the role of BabA and SabA adhesions in persistent infection and chronic inflammation . ,Umea university medical dissertations abstract no.83,ISSN 0345-7532,isbn91-7305-548-4.
15. Frezza M,Gorji N and Melato. (2001) :Histopathology of non –steroidal anti-inflammatory drug induced gastroduodenal damage; correlation with *H.pylori*,ulcers and haemorrhagic events .j.clinic.pathol.,54;521-525.
16. Court. M, Robinson. PA, Dixon. MF, Jeremy. AHT and Crabtree. JE. (2003):The effect of gender on Helicobacter felis mediated gastritis, epithelial Cell proliferation ,and apoptosis in the mouse model. Journal of Pathology.,201: 303–311.
17. Salomaa-Ra¨sa¨nen,A , Kosunen,T. Mattila, J S. Sarna, Sand H. Rautelin . (2004): Age-Dependent Accuracy of Helicobacter pylori Antibody Assays for Adults, with Special Emphasis on Atrophic Gastritis .,
18. Zhang C, Nobutaka Yamada, Yun-Lin Wu, Min Wen, Takeshi Matsuhisa, Norio Matsukura. (2005):Comparison of Helicobacter pylori infection and gastric mucosal histological features of gastric ulcer patients with chronic gastritis patients . World J Gastroenterol; 11(7):976-981
19. The Eurohepygast study group,(2002):Risk factors for atrophic chronic gastritis in European population.: results of Eurohepygast study. Gut; 50:779-785.

20. Figura N, Vindigni C, Presenti L, Carducci A . (1998):New acquisitions in Helicobacter pylori characteristics. Ital J Gastroenterol Hepatol,30 Suppl 3:S254-S258.

21. Hussein,N; Napaki S . (2009):Atherton J.A Study of Helicobacter Pylori-associated Gastritis Patterns in Iraq and Their Association with Strain Virulence. The Saudi Journal of Gastroenterology .. 15(2): 125-7

22. Malekzadeh R, Sotoudeh M, Derakhshan MH, Mikaeli J, YazdanbodA,Merat S, *et al*. (2004): Prevalence of gastric precancerous lesions in Ardabil: A high incidence province for gastric adenocarcinoma in the northwest of Iran. J Clin Pathol;57:37-42.

23. Fikret D, Kaya Ö, Suna E. (2001): Relationship between atrophic gastritis,intestinal metaplasia, dysplasia and Helicobacter pylori infection. Turkish J Gastroenterol;,12:169.

24. Ota H, Nakayama J, Momose M, Hayama M, Akamatsu T, Katsuyama T, Graham DY, Genta RM. (1998):Helicobacter pylori infection produces reversible glycosylation changes to gastric mucins. Virchows Arch.,433:419-426.

25. Segal ED, Cha J, Lo J, Falkow S, Tompkins LS. (1999): Altered states:involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. Proc. Natl. Acad. Sci. USA., 96:14559-14564

26. Aspholm M. (2004):Adaptation of Helicobacter pylori Adherence Properties in Promotion of Host Tropism and Inflammatory Disease.,UMEÅ University (Sweden) medical dissertations. New Series No. 87 ISBN: 91-7305-736-3.

27. Takenaka R, Yokota K, Ayada K, *et al*. (2004): *Helicobacter pylori* heat-shock protein 60 induces inflammatory responses through the Toll-like receptor-triggered pathway in cultured human gastric epithelial cells.*Microbiology*;150:3913–22.

28. Lee KH, Cho MJ, Yamaoka Y, *et al*. (2004):Alanine threonine polymorphism of *Helicobacter pylori* RpoB is correlated with differential induction of interleukin-8 in MKN45 cells. *J Clin Microbiol*; 42:3518–24.

29. Lin SN, Ayada K, Zhao Y, *et al*. (2005): *Helicobacter pylori* heat-shock protein 60 induces production of the pro-inflammatory cytokine IL8 in monocytic cells. *J Med Microbiol*. 54:225–33.

30. Satin, B., Del Giudice, G., Della Bianca, V., Dusi, S., Laudanna, C., Tonello, F. (2000) *:* The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. *J Exp Med*,191:1467-1476.

31. Slattery MJ, Dong C. (2003): Neutrophils influence melanoma adhesion and migration under flow conditions. Int. J. Cancer.,106:713-722.

32. Ismail, H; Fick,P; Zhang,J; Lynch,G† and Daniel J. (2003):Depletion of Neutrophils in IL-10$^{-/-}$ Mice Delays Clearance of Gastric Helicobacter Infection and Decreases the Th1 Immune Response to Helicobacter.

33. Olfat,O. (2003): Helicobacter pylori – Bacterial Adherence and Host Response. UMEÅ University medical dissertations ,(Sweden) . New Series No. ISBN 91-7305-537-9

34. Blaser MJ, Atherton JC.. Helicobacter pylori persistence: biology and disease. J. Clin. Invest. (2004),113:321-333.

35. Björkholm, B., Zhukhovitsky, V., Löfman, C., Hulten, K., Enroth, H., Block, M., Rigo, R.,Falk, P., Engstrand, L. (2000) *: Helicobacter pylori* entry into human gastric epithelial cells: A potential determinant of virulence, persistence, and treatment failures. *Helicobacter*,3:148-154.

36. Amieva, M.R., Salama, N.R., Tompkins, L.S., Falkow, S. (2002): *Helicobacter pylori* enter and survive within multivesicular vacuoles of epithelial cells. *Cell Microbiol* ,10:677-690.

37. Kwok, T., Backert, S., Schwarz, H., Berger, J., Meyer, T.F. (2002) *:* Specific entry of *Helicobacter pylori* into cultured gastric epithelial cells via a zipper-like mechanism. *Infect Immun*,4:2108-2120.

38. Dundon, W.G., Nishioka, H., Polenghi, A., Papinutto, E., Zanotti, G., Montemurro, P. *et al*. (2002) : The neutrophil-activating protein of *Helicobacter pylori*. *Int J Med Microbiol*,6-7:545-550.

39. Shimoyama, T., Fukuda, S., Liu, Q., Nakaji, S., Fukuda Y. and Sugawara. K. (2003):Helicobacter pylori water soluble surface proteins prime human neutrophils for enhanced production of reactive oxygen species and stimulate chemokine production . J Clin Pathol; 56:348–351

40. Mandell L, Moran AP, CocchiarellaA,Houghton J, Taylor N, Fox JG, Wang TC, Kurt-Jones EA.(2004) : Intact Gram-negative *Helicobacter pylori*, *Helicobacter felis*, and *Helicobacter hepaticus*bacteria activate innate immunity via Toll-like receptor 2 but not Toll-like receptor 4. *InfectImmun*;72:6446–54.

41. Chang YJ, Wu MS, Lin JT, Sheu BS, MutaT,Inoue H, Chen CC. (2004):Induction of cyclooxygenase-2 over expression in human gastric epithelial cells by *Helicobacter pylori* involves TLR2/TLR9 andc-Src-dependent nuclear factor-kappaBactivation.*Mol Pharmacol*;66:1465–77.

42. Viala J, Chaput C, Boneca IG, *et al*. (2004): Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. *Nat Immunol*;,5:1166–74.

43. Torok AM, Bouton AH, Goldberg JB. *Helicobacterpylori*induces interleukin-8 secretion by Toll-like receptor 2- and Toll-like receptor 5-dependent and -independent pathways. *InfectImmun*; .(2005), 73:1523–31.

44. Stagg AJ, Hart AL, Knight SC, *et al*. (2003):The dendritic cell: Its role in intestinal inflammation and relationship with gut bacteria.Gut: 52:1522.

45. Rachmilewitz D, Katakara K, Karmeli F, *et al* (2004):Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology: 126:520.

46. Cho,s;K. H. Kim,H .(2007):Extracellular signal-regulated kinase induces phosphorylation of IκBα in Helicobacter pylori-infected gastric epithelial AGS cells .Inflammopharmacology. 0925-4692 (Print) 1568-5608 (Online)PP.26-30

47. Cario E, Podolsky DK(2000): Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun ,68:7010.

48. Rosenstiel P, Fantini M, Brautigam K, *et al*. (2003):TNF-alpha and IFN-gamma regulate the expression of the NOD2 (CARD15)gene in human intestinal epithelial cells. Gastroenterology. 124:1001.

49. Fan, X. J., S. E. Crowe, S. Behar, H. Gunasena, G. Ye, H. Haeberle, N. Van Houten, W. K. Gourley, P. B. Ernst, and V. E. Reyes(1998):The effect of class II major histocompatibility complex expression on adherence of *Helicobacter pylori* and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1-mediated damage. J. Exp. Med.,187:1659–1669.

50. Jones, N. L., P. T. Shannon, E. Cutz, H. Yeger, and P. M. Sherman(1997):Increase in proliferation and apoptosis of gastric epithelial cells early in the natural history of *Helicobacter pylori* infection. Am. J. Pathol, 151:1695–1703.

51. Houghton, J., R. M. Korah, M. R. Condon, and K. H. Kim. (1999): Apoptosis in Helicobacter pylori-associated gastric and duodenal ulcer disease is mediated via the Fas antigen pathway. Dig. Dis. Sci.,44:465–478.

52. Katsikis, P. D., Wunderlich, E. S. ,Smith, C. A. and Herzenberg, L. A. (1995) :Fas Ag stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. J. Exp. Med.,181:2029–2036.

53. Giordano, C., G. Stassi, R. De Maria, M. Todaro, P. Richiusa, G. Papoff, G.Ruberti, Bagnasco, R. Testi, and A. Gassuzzo. (1997):Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. Science ,275:960–963.

54. Harris, P. R., H. L. Mobley, G. I. Perez-Perez, M. J. Blaser, and P. D. Smith. (1996) :Helicobacter pylori urease is a potent stimulus of mononuclear phagocyte activation and inflammatory cytokine production. Gastroenterology,111:419–425.

55. D'Elios, M. M., *et al*. (1997):T helper 1 effector cells specific for HP in the gastric antrum of patients with peptic ulcer disease. J. Immunol. 158:962–7.

56. Maekawa, K. (1997):Helicobacter pylori induces proinflammatory cytokines and HHC class II Ag in mouse gastric epithelial cells. J. Lab. Clin. Med.,130:442–449.