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Original Article

Role of Pro-inflammatory and Immunoregulatory Cytokines in Pathogenesis of Chronic Gastritis

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Summary:

Background: Chronic gastritis (CG) is histopathological entity characterized by chronic inflammation of the stomach that mostly caused by Helicobacter pylori, development of inflammation in gastric mucosa result in release of pro- and anti- inflammatory cytokines .This study aimed to shed light on the role of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF) in the development and prognosis of CG among Iraqi patients.

Patients and methods: 100 Iraqi patients with CG (61 male and 39 female) with age range (10-79) year, were involved in this stady while attending Specialist Hospital of Disease of Liver and Gastrointestinal System at Baghdad Medical City from Nov. 2007 to Apr. 2008. Patients divided according to histological diagnosis into three groups : 66 with active CG, 21 with Superficial CG and 13 with Inactive CG . All Patients were investigated for infection with H. pylori by histological examination and for quantitative estimation of serum anti H. pylori (IgG) by Enzyme Linked Immunosorbent Assay (ELISA), and Amplified Sensitivity Immuno Assay (EASIA) technique to measure the level of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF) for both patients and healthy control groups.

Results: Incidence of H. pylori is (66%) among all patients, highly significant increased ($p<0.01$) in serum level of anti H. pylori in patient groups: Active CG, Superficial CG and Inactive CG respectively and significant increased ($p<0.01$) trend of IFN-γ, IL-8, IL-4, IL-10, and GM-CSF in all patient groups as compared with healthy control.

Conclusions: High frequency of H. pylori in patient reflect the important role of H. pylori in etiopathogenesis of CG. Increased serum level of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF) propaply play crucial role in driving inflammatory process and promoting gastric mucosa destruction in CG, regulation of these cytokines is consider as an important therapeutic goal.

Key words: Chronic gastritis, IFN-γ, IL-8, IL-4, IL-10, GM-CSF.

Fac Med Baghdad
2010; Vol. 52, No. 3
Received Aug. 2009
Accepted Oct. 2009

Introduction:

Chronic gastritis is histopathological entity characterized by chronic inflammation of the stomach & it's classification based on the underling etiological agent (e.g, Helicobacter pylori , bile reflex, nonsteroidal anti inflammatory drugs) (1). H. pylori is gram-negative rods that have the ability to colonize and infect the stomach (2) the bacteria survive with the mucous layer that covers the gastric surface epithelium and the upper portions of gastric foveolae (3), H. pylori-induce chronic gastritis associated with an increase risk for the development of gastric cancer, this risk depends on the distribution & severity of gastritis (4). The interaction of H. pylori with surface mucosa result in the release of pro- and anti- inflammatory cytokines, which lead to recruitment of polymorphonuclear cells and may begin the entire inflammatory process (5, 6). Cytokines are regulatory proteins (8-60 KDa) secreted by white blood cells and variety of other cells in the body, the pleiotropic action of cytokines include numerous effects on cells of the immune system and modulation of inflammatory responses, multiple interaction between different individual cytokines including stimulating or inhibiting action(7). High levels of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF), are detected in gastric mucosa of patient with H. pylori CG (8) which indicate the important of these cytokines in regulate abroad range of inflammatory process that implicated in the pathogenesis of CG and progressive of chronic inflammation in mucosa,because IFN-γ play a pivotal role in tissue-damage (9,6) wherease IL-8 & IL-4 are attracted inflammatory cells (such as neutrophile, lymphocyte, Macrophage) to site of infection (10), on the other hand IL-10 is one of the most important mediators that physiologically limits and down-regulates inflammation (11), while the local expression of GM-CSF induce local inflammatory response in stomach infected with gastric inflammation (12).

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Patients and methods:

Atotal of 100 Iraqi patients with CG (61 male, 39 female) age ranged between 10-79 years were included in this study, ther diagnosis was based on the clinical, endoscopy and histological examination in Specialist Hospital of Disease of

Liver and Gastrointestinal System in Baghdad Medical City from Nov. 2007-Apr. 2008. Patients are divided according to histological diagnosis into three group, 66 with Active Chronic Gastritis (ACG), 21 with Superficial Chronic Gastritis (SCG), 13 with Inactive Chronic Gastritis (ICG). For comparative purposes, 30 healthy control individuals mached for age & sex were selected from healthy individuals that did not have symptoms of CG & were not taking any medication.

Methods: All patient groups were investigated for infection with H. pylori and tissue changes in the stomach by histological examination, staining the biopsies sections with H&E stain according to Bancroft and Stevens (1982) (13), and for quantitative estimation of serum anti H. pylori (IgG) using Enzyme Linked Immunosorbent Assay (Biohit plc, Helsinki, Finland). Moreover, Enzyme Amplified Sensitivity Immuno Assay techniques used to measure the serum level of IL-4, IL-10 and IFN-γ (Biosourse Europe S.A, Nivelles, Belgium), while the serum level of IL-8 and GM-CSF is measure by ELISA (Beckman Coulter Marseille, France) the serological assay were done for both patients and healthy control groups, and were conducted according to manufacturing company leaflet. Statistical analysis was assessed using Spss Version 10 (Software Statistical Package for Social Science), Statistical significance was determined using L.S.D. test for quantitative datd, correlation is considered significant when probability valve ≤0.05. Results were expressed as percentage, mean±S.D. (14).

Results:

Histological examination revealed the incidence of H. pylori is (66%) among all patients, while serological examination showed increased in the number of patients infected with H. pylori (70%), which was reflected by highly significant increased ($p<0.01$) in serum level of anti H. pylori in patient groups: ACG, SCG and ICG respectively in comparison with control group as clearly shown in table -1. All patient groups ACG, SCG and ICG revealed significant increased ($p<0.01$) in serum level of IFN-γ, IL-4 & GM-CSF as compared with control group. Statistical analysis by using L.S.D. test showed no significant difference when compared patient groups with each other, as illustrated in table-2,3,4. Significant elevation ($p<0.01$) in serum level of IL-8 in all patient groups when compared with control group. L.S.D. test revealed significant difference between SCG v.s ICG also between ACG v.s ICG, table-5. Moreover, level of IL-10 was raised in all patient groups in comparison with control group, L.S.D. test revealed significant difference when compared ACG with SCG, table-6.

Table 1: Serum level of IgG anti-H. Pylori (EIU) easured by ELISA in patients with CG and control group.

Study groups	No.	Level of IgG antibody Anti-H. pylori (EIU) Mean ± standard division	ANOVA
chronic Active (ACG) gastritis	66	48.65 ± 23.99	High statistical ($p<0.01$)
Superficial chronic gastritis (SCG)	21	43.09 ± 26.99	
Inactive chronic gastritis (ICG)	13	39.15 ± 27.78	
Control group	30	22.46 ± 8.82	

Statistical analysis between study groups			
Study groups		L.S.D. P-value	Statistical
Control group	ACG	0.00	H.S
	SGC	0.002	H.S
	ICG	0.027	S
ACG	SCG	0.325	N.S
	ICG	1.66	N.S
SCG	ICG	0.620	N.S

Table 2: Serum level of IFN-γ (UI/ml) measured by EASIA in patients with CG and control group.

Study groups	No.	Level of IFN-γ (UI/ml) Mean ± standard division	ANOVA
gastritis chronic Active (ACG)	66	7.30 ± 4.55	High statistical ($p<0.01$)
Superficial chronic gastritis (SCG)	21	6.75 ± 1.73	
Inactive chronic gastritis (ICG)	13	7.51 ± 4.05	
Control group	30	4.09 ± 1.58	

Statistical analysis between study groups			
Study groups		L.S.D. P-value	statistical
Control group	ACG	0.00	H.S
	SGC	0.012	S
	ICG	0.006	H.S
ACG	SCG	0.549	N.S
	ICG	0.852	N.S
SCG	ICG	0.558	N.S

Table 3: Serum level of IL-4 (pgm/ml) measured by EASIA in patients with CG and control group

Study groups	No.	Level of IL-4 (pgm/ml) Mean ± standard division	ANOVA
Active chronic gastritis (ACG)	66	246.24 ± 201.58	High statistical (p<o.o1)
Superficial chronic gastritis (SCG)	21	244.38 ± 180.57	
Inactive chronic gastritis (ICG)	13	262.51 ± 233.20	
Control group	30	87.75 ± 48.14	

Statistical analysis between study groups

Study groups		L.S.D. P-value	L.S.D. statistical
Control group	ACG	0.00	H.S
	SGC	0.003	H.S
	ICG	0.004	H.S
ACG	SCG	0.967	N.S
	ICG	0.764	N.S
SCG	ICG	0.774	N.S

Table 4: Serum level of GM-CSF (pgm/ml) measured by ELISA in patients with CG and control group.

Study groups	No.	Level of GM-CSF (pgm/ml) Mean ± standard division	ANOVA
Active chronic gastritis (ACG)	66	118.95 ± 84.89	High statistical (p<o.o1)
Superficial chronic gastritis (SCG)	21	115.98 ± 58.65	
Inactive chronic gastritis (ICG)	13	83.47 ± 46.0	
Control group	30	66.03 ± 28.60	

Statistical analysis between study groups

Study groups		L.S.D. P-value	L.S.D. statistical
Control group	ACG	0.001	H.S
	SGC	0.001	H.S
	ICG	0.011	S
ACG	SCG	0.863	N.S
	ICG	0.089	N.S
SCG	ICG	0.179	N.S

Table 5: Serum level of IL-8 (pgm/ml) measured by ELISA in patients with CG and control group.

Study groups	No.	Level of IL-^ (pgm/ml) Mean ± standard division	ANOVA
Active chronic gastritis (ACG)	66	282.86 ± 185.85	High statistical (p<o.o1)
Superficial chronic gastritis (SCG)	21	321.07 ± 139.41	
Inactive chronic gastritis (ICG)	13	384.12 ± 177.56	
Control group	30	144.96 ± 55.07	

Statistical analysis between study groups

Study groups		L.S.D. P-value	L.S.D. statistical
Control group	ACG	0.00	H.S
	SGC	0.00	H.S
	ICG	0.00	H.S
ACG	SCG	0.333	N.S
	ICG	0.035	S
SCG	ICG	0.257	N.S

Table 6: Serum level of IL-10 (pgm/ml) measured by EASIA in patients with CG and control group.

Study groups	No.	Level of IL-10 (pgm/ml) Mean ± standard division	ANOVA
gastritis chronic Active (ACG)	66	228.94 ± 260.93	High statistical (p<o.o')
Superficial chronic gastritis (SCG)	21	67.67 ± 36.48	
Inactive chronic gastritis (ICG)	13	170.85 ± 235.99	
Control group	30	53.51 ± 22.39	

Statistical analysis between study groups

Study groups		L.S.D. P-value	L.S.D. statistical
Control group	ACG	0.00	H.S
	SGC	0.806	N.S
	ICG	0.082	N.S
ACG	SCG	0.002	H.S
	ICG	0.345	N.S
SCG	ICG	0.150	N.S

Discussion:

Many studies reported abroad have mentioned that there is a strong associated between chronic infection caused by H. pylori and chronic gastritis, moreover it>s the most common cause of chronic gastritis (15), and could be found in spiral shape or curve rods adhesion to the mucus layer of antrum (5). In present study serological examination showed higher sensitivity in diagnosis of bacteria than histological examination, this may be due to difficult recognize of the bacteria in tissue specially

in sections contain few number of bacteria (16), the presence of H. pylori associated with tissue damage & histological finding by initiating of chronic inflammation in gastric mucosa, this inflammation is mediated by an array of pro- and anti- inflammatory cytokines (17). Significant increase in concentration of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF) in patient groups providing evidence that these cytokines play crucial role in immune and inflammatory responses in chronic gastritis coincine with previous world wide studies (10). Cytokines interact in complex manner in development & progression of an inflammatory environment in which IFN-γ is the most predominant Th1 cytokine produced in chronic gastritis induced by H. pylori which plays a pivotal role in both protection and tissue-damaging gastritis (3), apart from it's effects on mucosal immunity, IFN-γ has been suggested to stimulate gastric epithelial cell apoptosis, by promoting the production of nitric oxide (NO), or by enhancing the attachment of bacteria to gastric epithelia, whether a protective function of IFN-γ is help to eliminate the invading bacteria and minimize mucosal cell injury (6). Whereas IL-8 is responsible for the maintenance of chronic inflammation in gastric mucosa by driving the chemotaxis of inflammatory cell at infected mucosa (10) and the levels of IL-8 are in parallel with the histological severity of gastritis, high level of IL-8 may also be associated with increased risk of malignancy (such as gastric carcinoma) (18). IL-10 is an immunoregulatory Th2 cytokines that may play arelevant role in the H. pylori-induced immune response (19), although that may limit inflammatory response (8), the IL-10 production triggers the immune escape mechanisms of H. pylori by generating type 1 regulatory T cell (Tr-1 cells) (19). Many studies indicate that expression of IFN-γ (aTh 1 cytokine) enhanced gastric inflammation, whereas expression of certain Th 2 cytokines (IL-10 and possibly IL-4) contributes to diminshed inflammation (8). GM-CSF & IL-4 are important mediators in Th 2 host response to infection through their ability to prevent and delayed apoptosis of PMNs and GM-CSF & IL-4 effect on the maturation and activation of neutrophils, eosinophils & dentritic cells which are necessary in microbial resistance (20), furthermore, GM-CSF stimulate granulocyte proliferation & maturation (12). While IL-4 may be cotribute in develop of histological & physiological changes in gastric mucosa because it's cause atrophy & metaplasia in goblet cells in gastric epithelium (9).

Conclusion:

Increase level of cytokines (IFN-γ, IL-8, IL-4, IL-10, and GM-CSF) play role in driving inflammatory process and promoting gastric mucosa destruction in CG regulation of these cytokines is considered as an important therapeutic goal.

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