Increase serum IL-8 Level in Iraqi Patients with *Helicobacter Pylori Cag*A Genotype Infection

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ABSTRACT:

BACKGROUND:

More than half of all humans are colonized in their stomachs by *Helicobacter pylori* . That carriage was nearly universal among adults in developing countries suggests that in earlier times most humans carried these organisms.*H. pylori* was an important human pathogen that causes gastritis and is strongly associated with gastric ulcers, gastric adenocarcinomas, and mucosa-associated lymphoid tissue lymphomas. In response to *H. pylori*, interleukin-8 (IL-8) was secreted from host cells to attract components of the innate and adaptive immune systems to the site of infection.*Cag*A^{+} cells induce higher levels of the proinflammatory IL-8 cytokine. That such effects are specific for *Cag*A island genes had been shown by our study.

OBJECTIVE:

To determine the role of incidence of *H.pylori CagA* positive strain in induce higher level of the proinflammatory cytokine IL-8.

METHODS:

One hundred and fifty(150) Patients attended the Endoscopic Unit at"Gastroenterology and Hepatology Teaching Hospital/ Baghdad Medical City"were included in this study with ages range from 18 years to 65 years . The source of specimens to undergo oesophageal gastroduodenoscopy (OGD) collected from April 2009 to end of March 2010 were eligible for this study.Two types biopsy of samples for histopathology to detect *H.pylori* and detection of *Cag*A gene by *In Situ* hybridization(ISH) and blood samples for estimation of serum IL-8 level by ELISA.

RESULTS:

There was a significant increase in IL-8 serum levels that $P<0.05$ due to the significant increase of *Cag* A in *H.pylori* positive strains.

CONCLUSION:

Our study revealed that increased in *Cag*A expression in *H.pylori* positive strains and induced higher levels of the proinflammatory cytokines .

KEY WARDS:*h.pylori*,IL-8, *cag A*.

INTRODUCTION:

Helicobacter pylori possessing the *Cag* A(cytotoxin-associated gene) pathogenicity island that had been shown to have increased virulence, to cause higher levels of mucosal inflammation. *Cag* A cytotoxin appeared to play a vital role in disease outcome[1,2].

The *Cag*A (cytotoxin-associated gene A) was so-named because it was thought to be associated with expression of the vacuolationg cytotoxin *Vac*A. However, the *Cag*A gene was not chromosomally linked to the *Vac*A gene; nor was it needed for expression of *Vac*A[3].The *Cag*A gene was present in strains with enhanced virulence, and had been identified as an important risk factor for development of severe gastric disease. *Helicobacter pylori* strains were divided into two groups named type I or type II strains, based on whether or not they express *Cag*A[4]. Interestingly, not all the *H. pylori* infected individuals develop peptic ulcer or gastric cancer. A significant number of patients had milder form of

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disease, like inflammation in stomach and duodenum[5]. Such discriminatory behavior of *H. pylori* could be linked to the ability or inability of the bacteria to express various virulence factors. These virulence factors helped *H. pylori* in adapting to its environment . The expression of cytotoxin-associated gene (*Cag*A and/or *Vac*A) and cytotoxin production by *H. pylori* strains had been studied and correlated with their enhanced pathogenic potential[6] .

The Interleukin IL-8 was a potent neutrophil and lymphocyte-activating chemotactic cytokine (chemokine), and gastrointestinal epithelial cells secreted biologically activated IL-8 in response to infection with pathogenic bacteria[7].Chemokines produced by activated enterocytes bind to the extra cellular matrix, thereby establishing a chemotactic gradient that directs inflammatory cell migration toward the epithelial cell surface[8] . Human neutrophils could produce IL8 that play an important role in the pathology of *H. pylori* associated gastritis, which is characterised by chronic neutrophil infiltration. Neutrophils were a major source of oxygen derived free radicals[9] .*Cag*A positive *H. pylori* stimulated neutrophils could be relevant in gastric mucosal damage via the generation of reactive oxygen metabolites[10,11] and the released of proteolytic enzymes[12].*Helicobacter pylori–cag*$^{+}$ cells induced higher levels of the proinflammatory cytokines IL-1β and IL-8. That such effects are specific for *Cag*A island genes had been shown by mutation studies.

PATIENTS AND METHODS:

One hundred and fifty(150) patients attended the Endoscopic Unit at "Gastroenterology and Hepatology Teaching Hospital/ Baghdad Medical City"were includ in this study with ages ranging from 18 years to 65 years .The source of specimens to undergo was oesophageal gastroduodenoscopy (OGD) from April 2009 to end of January 2010 were eligible for this study. They were all suffering from clinical manifestation of gastroduodenal ulcer and /or gastritis.The diagnosis was based on the clinical and endoscopy examination under supervision of physicians or surgeon specialists.

Patients were excluded from the study if they taking a proton pump inhibitors, taking H2-blockers and within past 2 weeks had received bismuth compounds, antibiotics, or eradication therapy for *H.pylori* and in case of administration of non-inflammatory drugs (NSAID).

At least two antral and three corpus biopsy specimens were collected from each patient. A total of two antral and two corpus biopsy specimens were used for histological analysis and for mRNA extraction by *In Situ* hybridization for detection *Cag*A gene.

From each patient 5 ml blood was collected .All fasting blood was taken before endoscopy .Serum samples were obtained for detection IL-8 by Biosource enzyme immunoassay Kit which provided materials for the quantitative determination of IL-8 in serum by Sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) (as recommended by the manufacturer company).

Statistical analysis:

Data were translated into a computerized database structure .An expert Statistical advice was sought for Statistical analysis and was done using SPSS version 17 computer software (Statistical package for social sciences).

RESULTS:

A total of one hundered fifty(150) Iraqi patients distributed as females 56(43.1%)and males 74(56.9%)with GIT symptoms distributed as one hundered thirty(130)suffered from gastritis,gastric ulcer and duodenal ulcer caused by *H.pylori* termed as (HP+ve) and twenty patients termed as HP-ve compared with twenty healthy control distributed as females 9(45.0%)and males 11(55.0%) there was no significant differences between females and males according to chi-square test . Age range (<20 to=>60 years)that distributed as <20years counted 4(3.1%),20-29 counted 26(20%),30-39 counted 29(22.3%),40- 49counted 24(18.5%),50-59counted as(19.2%) and=>60 as 22(16.2%) ,while the healthy controle group(20) distributed as <20 counted 1(5.0%),20-29counted 5(25.0%),30-39counted as 4(20.0%),40-49counted as 7(35.0%),50-59counted as2(10.0%)and=>60counted1(5.0%)there was no significant differences(5.396) between age groups(table1).

Table 1: Age and gender distribution in *H.pylori* infection

		Groups			
		GIT		Control	
		N	%	N	%
Gender	Male	74	56.9%	11	55.0%
	Female	56	43.1%	9	45.0%
Age (years)	<20	4	3.1%	1	5.0%
	20--29	26	20.0%	5	25.0%
	30--39	29	22.3%	4	20.0%
	40--49	24	18.5%	7	35.0%
	50--59	25	19.2%	2	10.0%
	=>60	22	16.9%	1	5.0%
Total		130	100%	20	100%

SerumIL-8 level *and* *H.pylori* *Cag*A positive infection:

By *Insitu* hybridization(ISH)detection of *Cag*A mRNA expression as positive (+ve) strain and if the strain of *H.pylori* not expressed *Cag*A refered as negative strain(- ve) our study revealed that *Cag* A positive strain were 84 (76.4%) and the *Cag*A negative strain were 26 (23.6%) from a total of 110 HP positive patients and there is significant increase in serum IL-8 concentration (P<0.05) in association with *Cag*A expression in *H.pylori* positive patients as shown in table(2 and 3).

Table 2 :Prevalence of *Cag*A expression in *H.pylori* positive patients

Cases	*Cag*A(detected in *H.pylori* positive patients only)					
	GIT			Healthy control and HP-ve		
		Count N%	Total HP+ve			
*Cag*Aexpression	Positive	84 76.4%	110	-	-	-
	Negative	26 23.6%		-	-	-

Table3: Increase serum IL-8 in association with *Cag*A

Cag A expression			
Positive		Negative	
Mean±SEM (Min-Max)		Mean±SEM (Min-Max)	
IL-8 (pg/ml)	235.218±21.710 (54.054-908.580)	156.946±11.851 (36.910-302.245)	0.050*

DISSCUSSION:

In the present study it was found that there was no difference between males and females and between age groups for acquisition of *H. pylori* infection , this result is in agreement with the finding of Sedlackova et al (2003) and Caballero et a.l(1997) [13], this may indicate that there was no differences in genetic factor or other factors that influence the colonization or adherence of *H. pylori* at gastrointestinal tract wall. IL-8, a potent T- cell and neutrophil chemoattractant and activity agent, was considered

an important factor in the pathogenesis of inflammatory disease and it was an important cytokine in the host inflammatory response to *H. pylori*[7,8] , which correlated with its induction in gastric epithelial cell cocultured with *H. pylori* in vitro[14] , up-regulation of IL-8 by *H. pylori* might lead to free radical generation and the release of proteolytic enzymes from activated neutrophils affecting mucosal integrity[15].In this study the serum level of IL-8 IL-8 higher in *H.pylori* infected patients(mean =235.218±21.710 pg/ml) than control,IL-8 higher in H.pylori infected patients ($P<0.05$) our study matched with Yamoaka et al.(1996) [16] that showed *H. pylori* infection was associated with increase expression and production of IL-8($P<0.0001$).Increasing of IL-8 level in *H.pylori* patients serum was due to the chemotactic activity of IL-8 for neutrophils to infiltrate in infected area colonized with *H. pylori* and this lead to inflammatory reaction which appeared as a gastric or duodenal pain,and on the other hand, IL-8 production relates to *H. pylori* density and *Cag*A+ which also affect on the severity of symptoms[16].*Cag*A positive *H pylori* strains had been known to be associated with severity of disease outcome[17].The results obtained from our study were consistent with results of the previous study showed a significant increase of serum IL-8 level induced by *Cag*A isolated from biopsy($P<0.05$) .The time at with the infection was contracted which important, especially because *H. pylori* was known as a life-long infection and it colonize the human stomach for a long period of time until it cause severe infections[18].This explain that *H.pylori Cag*A^+ cells induced higher serum levels of the proinflammatory IL-8.That such effects were specific for *Cag*A island genes had been shown by our studies.

CONCLUSION:

Our study revealed that increased in *Cag*A expression in *H.pylori* positive strains and induced higher serum level of proinflammatory IL-8.

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