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Investigation of IgG and IgM seroprevalence of *Helicobacter pylori* infections and their relation toIL-6 and some risk factors among dyspeptic patients in Al-Qasim city of Babylon province

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ABSTRACT

We aimed to explore the predominance of *H. pylori* infections in dyspeptic patients by detection specific IgG and IgM and their relationship to IL-6 and some risk factors. A total of 250 blood specimens were collected from patients with dyspeptic symptoms. ELISA technique was utilized to detect specific anti-*H. pylori* IgG and IgM, and IL-6 concentration. Results showed high IL-6 concentrations in seropositive compared with seronegative dyspeptic patients group at probability level ($P \leq 0.005$). 201 (80.4%) of the sera specimens were positive to *H. pylori*(IgG), 115 (46%) were positive to *H. pylori* (IgM),95 (38%) were positive to both IgG and IgM, and 29 (11.6%) were negative to both .The prevalence rates of IgG and IgM were higher in male 87.4% (97/111) and 53.2%(59/111) than female 74.8 (104/139) and 40.3% (56/139) respectively. Additionally, the age of 51-60 years had the highest (93.8%) *H. pylori*-IgG prevalence rate, while *H. pylori*-IgM (62.9%) was predominant among patients in the age group 41-50 years. *H. pylori* IgG and IgM prevalence rates were 93.9% (92/98) and 64.3 %(63/98) respectively among smokers. In conclusion, the high infection rate of *H. pylori* in dyspeptic patients was significantly associated with IL-6, and prevalence rate of *H.pylori* infection strongly correlates with gender, age, smoking and presence of *H.pylori*-infected family members.

Keywords: *Helicobacter pylori*, dyspeptic patients ,sero-prevalence, IgG, IgM, IL-6

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INTRODUCTION

Dyspepsia is a widespread health problem that defined as a complicated group of symptoms, instead of indicating a precise disease. Numerous causes of dyspepsia were identified, including *Helicobacter pylori*, which may also generate diverse symptoms in different patients(1), (2).Most of people with dyspepsia do not have any organic disease, but some of them are diagnosed with peptic ulcers and can be treated -particularly, those whose ulcers are associated with *H. pylori* infections (3). *H. pylori* is a frequent etiological agent worldwide, and it is described as gram-negative, microaerophilic, and spiral microorganism. It is a considerable cause of some health problems including, peptic ulcers, chronic active gastritis, and some stomach illnesses associated with lymphoma and gastric carcinoma(4). *H. pylori* is categorized as a first-class carcinogen(5), (6).

H. pylori specifically colonizes the gastric mucous membrane of more than one half of populations with high occurrence in developing countries(7),(8). Many factors may affect the frequency of of *H.pylori* infections, and these factors include: aging, previously infected family members, environmental hygiene, poor individual, weak socioeconomic state, and low living standards. It is typically acquired during childhood, and may persist for the life span if not treated (9),(10). Most of the *H. pylori* infections occur without noticeable symptoms(5). Although humoral and cellular immune responses are strongly provoked by *H. pylori* at the local and systemic level(11), hosts are incapable to eliminate the bacterium. The immune cells pervade the mucosa when *H. pylori* colonizes the stomach. The molecular mechanisms of initiation local immune response by this bacterium is complicated. It is supposed that the cytokines produced by immune and non-immune cells increases the enduring inflammation(12).

H. pylori infection can be diagnosed by either invasive or non-invasive tests (13). Infected people by *H. pylori* have specific IgG, IgA and IgM antibodies circulating in their blood. Recognition of specifiedanti-*H. pylori* antibodies has been employed as non-invasive serology method to detect *H. pylori* infections(14). Determining of IgG antibodies for *H. pylori* is now considered as the only indicator for a persistent infection of this bacterium, while identifying IgM antibodies is the more precise indicator for a newly obtained infection by *H. pylori* (15).However, only few reports have been published in Iraq to explore the predominance of *H. pylori* infections among dyspeptic patients by detecting both IgG and IgM; there is no similar study in Babylon province. In addition,there is no report to measure IL-6 levels among *H. pylori*-infected dyspeptic patientsin Iraq. Therefore, this study was designed to:

1. Explorethe prevalence of *H. pylori* infections amongst dyspeptic patients by detection specific anti-*H.pylori* IgG and anti-*H.pylori* IgM antibodies by ELISA.
2. Assess IL-6 concentrations among dyspeptic patients by ELISA.
3. Determine the association between *H. pylori* infections and some risk factors.

MATERIALS & METHODS

1-Patients: A total of 250 blood specimens were obtained from patients with dyspeptic symptoms who consulted private clinics at Al-Qasim city of Babylon province between January,2015 to January,2016. Every patient was asked to answer a prepared questionnaire to provide information about age, gender, habitation,smoking and family history for the presence of *H.pylori* infection.

2- Serological study: every serum sample was separated from blood and kept at –20º to be used(16). Anti-*H. pylori* IgG and IgM antibodies were detected in sera by using ELISA kit provided from Diagnostic Automation, inc.,(USA). IL-6 was assayed using ELISA kit provided from Ray Biotech Company (USA).

3-Statistical analysis: Statistical package for social sciences (SPSS version 23) was used for data analysis. In one direction (ANOVA one way) was used to search the existence of differences between IL-6 concentrations at the level of probability ($P \leq 0.005$) and results proven as arithmetic mean ± standard deviation. Chi-square was also employed for assessment of frequencies.The P-value of < 0.05 was regarded as significant(17).

RESULTS

This study was included 250 patients with dyspeptic symptoms. All of them were selected and subjected to clinical examination by senior physician. From the screened 250 sera specimens, 201 (80.4%) were positive to*H. Pylori* immunoglobulin G (IgG), 115 (46%)were positive for *H. pylori* immunoglobulin M (IgM),95 (38%) were positive to both IgG and IgM, and 29 (11.6%) were negative to both (Figure 1).

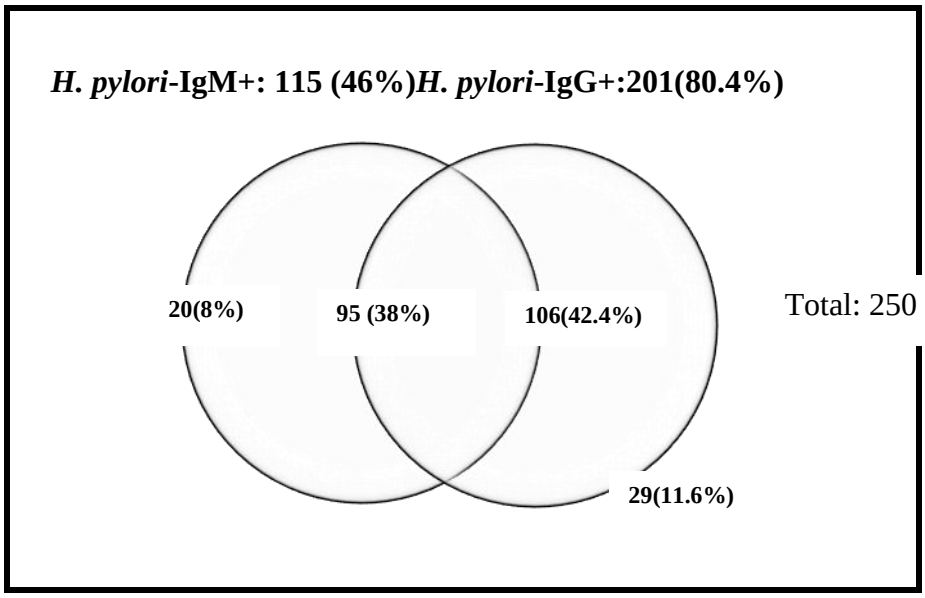

Figure. 1 **Specific anti *H. pylori* IgG and IgM antibodies seroprevalence among dyspeptic patients**

The study recorded higher significant serum IL-6 concentration at completely and partially different seropositive *H. pylori* infected patients groups, comparing with completely seronegative patients group at probability level (P ≤ 0.005). It was recorded (284.76±29.66) in seropositive *H. pylori* IgG and seronegative IgM group, and the concentration was (218.42±36.65) in seropositive *H. pylori* IgG and seropositive IgM group. The results also showed higher concentration (234.55±25.47) in seronegative *H. pylori* IgG and seropositive IgM group, while IL-6 concentration was (69.86±14.5) in seronegative *H. pylori* IgG and seronegative IgM group (Table 1).

Table.1 Concentration of IL-6 (Pg/ml) in patients groups

Patients groups	Number	Rang of IL-6concentrations(Pg/ml) M±S.D.
Hp. IgG (+ve)+Hp. IgM (-ve)	106	284.76±29.66[a]
Hp. IgG (+ve)+Hp. IgM (+ve)	95	218.42±36.65[a]
Hp. IgG (-ve)+Hp. IgM (+ve)	20	234.55±25.47[a]
Hp. IgG (-ve)+Hp. IgM (-ve)	29	69.86±14.5[b]

*** a and b letters indicate significant differences at (P ≤ 0.005).**
*** Hp: *H. pylori***

The results showed significant association between gender and *H. pylori* infections as shown in (Table 1). The prevalence rates of IgG and IgM were higher in male 87.4% (97/111),53.2%(59/111) when compared to the female 74.8 (104/139), 40.3% (56/139) patients respectively. In relation to age the results showed significant association between the age and *H. pylori* infection with the highest (93.8%) *H. pylori*-IgG prevalence rate at age of 51-60 years, while the maximum prevalence for *H. pylori*-IgM (62.9%) was recorded within the age group 41-50 years. *H. pylori* infections was statically not associated with type of residence (IgG: χ2=3.397, p=0.065), (IgM: χ2=0.133, p=0.715). However the study recorded high prevalence of 86.9% (73/84) *H. pylori* IgG among patients living in rural. The results showed a strong significant association between smoking and *H. pylori* infection with higher significant prevalence of 93.9% (92/98)*H. pylori* IgG, and 64.3 (63/98) for IgM among smokers. Prevalence of IgG antibody for *H. pylori* infection was significantly related to

the occurrence of familial infection (χ2=8.361, p=0.004), whereas that of *H. pylori*-IgM was not statistically significant (χ2=0.340, p=0.560) (Table2).

Table.2 *H. pylori* seropositivity in dyspeptic patients according to the demographic characteristics

Variables		Total	Hp. IgG (+ve) (%)	χ2	P-value	Hp. IgM (+ve)(%)	χ2	P-value
Gender	Male	111	97 (87.4%)	6.185	0.013*	59 (53.2%)	4.112	0.043*
	Female	139	104 (74.8%)			56 (40.3%)		
Age groups	10-20	42	26 (61.9%)	17.629	0 .007*	19 (45.2%)	16.865	0.010*
	21-30	63	56 (88.9%)			37 (58.7%)		
	31-40	51	41 (80.4%)			20 (39.2%)		
	41-50	35	29 (82.9%)			22 (62.9%)		
	51-60	32	30 (93.8%)			10 (31.3%)		
	61-70	19	13 (68.4%)			4 (21.1%)		
	71>	8	6 (75%)			3 (37.5%)		
Residence	Urban	166	128 (77.1%)	3.397	0.065	75 (45.2%)	0.133	0.715
	Rural	84	73 (86.9%)			40 (47.6%)		
Smoking	Smoker	98	92 (93.9%)	18.579	0.000*	63 (64.3%)	21.697	0.000*
	non smoker	152	109 (71.7%)			52 (34.2%)		
Family history	+	157	135 (86%)	8.361	0.004*	70 (44.6%)	0.340	0.560
	-	93	66 (71%)			45 (48.4%)		
Total		250	201 (80.4%)			115 (46%)		

Key: Hp: *H. pylori,* χ 2=Chi-square, (p<0.05) * = Significant association exist

DISCUSSION

Dyspepsia can be caused by many reasons including ,microscopic inflammation, psychiatric illness, motor abnormalities as well as *Helicobacter pylori* infection,which considered the most probable cause. It has been shown in international reports the high incidenence of *H. pylori* infection in dyspepsia patients with abnormalities in gastric mucosal(18). Serologic tests offer a fast and some how easy way to diagnose *H. pylori* infections,and these tests are not likely to show false negative results in people recently treated with bismuth compounds or omeprazole (19).

In this study, the overall prevalence of (80.4%) and (46%) for *H. pylori* IgG and IgM antibody among dyspeptic patients was nearly consistent to similar local study conducted in Baghdad city and reported prevalence of (80%) for *H.pylori* IgG but (59.2%) for IgM antibody among dyspeptic patients(20), and compatible with a study in Baquba city, which showed (80%) seroprevalence of anti-*H.pylori* IgG among randomly selected individuals(21). This is probably because of the similarity in living conditions and similar environmental factors (22), wheres *H. pylori* IgG prevalence in our study was higher than (64.8%) which recorded by a study on dyspeptic patients attended outpatient clinic in Erbil city(23). In neighboring countries, a study on randomly selected Turkish patients referred from an outpatient clinic recorded prevalence of

(50.5%) for *H.pylori* IgG(24). Additionally, epidemiological survey on *H. pylori* infection revealed that the infection prevalence in developing countries ranged between 7 and 87% and was lower in European(25).

A persistence infection of *H. pylori* stimulates the responses ofspecific B and T cells. Finding specified anti *H. Pylori*IgG is regarded an accurate way to diagnose *H. pylori* infections(26).However, *H. pylori* IgG levels can remain high for up to six months in blood even after eradication; thus it is difficult to distinguish between past and recent infections(27).So, the presence of IgM antibodies to *H. Pylori* without high specific IgG antibody titer is regarded as a serologic marker of primary *H. pylori* infection.On the other hand, increased IgM and IgG antibodies reflects activation of a chronic infection or reinfection by*H. Pylori*(28).

The seronegativity of remaining IgG seropositive patients with dyspeptic symptoms to *H. pylori*-IgM suggests that their blood samples might have been collected after the acute IgM seroconversion, which indicates a chronic infection(28).On the other hand, seronegativity to both *H. pylori*-IgG and IgM may be explained by other causes to dyspeptic symptoms rather than bacterial infection(29).

Stimulation of the gastric mucosa inflamation is the major pathophysiological incident in *H. pylori* infection, and this process is mediated and controlled by inflammatory cytokines generated by epithelial cells in response to this bacterium (30). Acute and chronic *H.pylori* infections are significantly correlating to the anti-inflammatory and proinflammatory cytokine, Interleukin-6 (IL-6)(31). Its anti-inflammatory influence occurs by means of the suppression of TNF-a and IL-1.(32). Our study revealed association between serum IL-6 levels and *H. pylori*-infected dyspeptic patients, which was reflected in significantly increased level of serum IL-6concentrations in different seropositive *H. pylori*-infected dyspeptic patients groups comparing with completely seronegative dyspeptic patients group, and this is consistent with the finding of KabirandDaar(33),which showed higher levels of IL-6 ingastritis patients infected by*Helicobacter* compared with Helicobacter-negative groups.

Our study showed a significant association between *H. pylori* infection and gender with a higher *H. pylori* prevalence rate in males than females for both IgG and IgM antibodies. This finding was close to that of Yasir *et al*., (2014)(18) and Valliani *et al*. (2013)(34) who indicated that *H. pylori* sero-positivity occurred more commonly in male than that of in female. This observation may be explained by the stress and work impact to which men more often expose to than women(35).

The prevalence of seropositive *H. pylori* IgG among dyspeptic patients increased with age, being maximum (93.8%) at age group 51-60 years. This agrees with the finding of Ajiboye*et al*., (2016)(36) who reported that the fifth decade of age was the peak of infection among dyspeptic patients. However, the peak of *H. pylori* IgM seropositivity rate (62.9%) was noted among those within 41-50 years of age, and this is may be because younger people are at higher risk of *H. pylori* infection although symptoms may not be significant in most individuals until later in adulthood and often only after long periods of latency(9),(37). Moreover, this may be due to effect of lower prostaglandinsconcentration, which are less produced with increased in age, and at the end reflected on gastric acid and on the integrity of the mucous membrane of the gastrointestinal tract leading to an increase in the infection of *H. pylori* bacteria(38).

The seroprevalence of *H. pylori*infection among rural residency was high when compared to that of urban, which can be explained by factors that facilitate acquisition of infection, such as inadequate living resources and poor sanitation(39).The test of association between residency and *H. pylori* IgG and IgM seropositivity revealed no significant association ($p>0.05$).

The current study also revealed that smoking was highly correlated to *H. pylori* infection with high prevalence among smokers. Nicotine may affect the secretion of mucus, the flow of the gastric mucous in the blood, and the production of the epidermal growth factor that may assist colonization when exposing to bacteria. Furthermore, the result may be due to of the socioeconomic situation, education state, and health conditions.(39)

The significance of relative contact is confirmed by the significant correlation ($p<0.05$) that noted between family history and the prevalence of *H. Pylori* IgG infection. The highest incidence rate of (86%) was recorded among those who have family history for *H. pylori* infection, and this is consistent with the finding that *H. pylori* transmitted mainly among the family setting.(40)

CONCLUSION

Our report revealed that *H. pylori* infection rate is high in dyspeptic patients, and IL-6 was significantly associated with*H. pylori*infecteddyspeptic patients.*H. pylori* infections were significantly related tosex, age, smoke and existence of *H.pylori*-positive family members. Furthermore, no significant association between *H. pylori* infections and type of residence in dyspeptic patientshas been indicated.

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