

Evaluation the relationship between the antibodies of *Helicobacter pylori* and Interleukin-6 in patients with gastritis in Kirkuk province

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Abstract

Background: *Helicobacter pylori* is recognized as the most common cause of chronic gastritis, and also an important pathogenic factor in peptic ulcer disease, after colonization and persistent in the mucus layers of the human stomach causes a raised level of pro-inflammatory cytokine such as IL-6.

Materials and Methods: The study was conducted in Kirkuk city from 15th of December 2016 to 15th of June 2017 in Azady teaching hospital in Kirkuk governorate, anti *H. pylori* IgG level and IL-6 level were estimated by using anti *H. pylori* IgG ELISA kit and IL-6 ELISA kit.

Results: A total of 90 patients the interleukin-6 level and anti *H. pylori* IgG level in a group of untreated patients against *H. pylori* infection (225.592 ng/L, 1.338 pg/ml respectively) and in a group of patients who had previously treated against *H. pylori* infection (102.60 ng/L, 0.350 pg/ml respectively) were significantly higher as compared to those of the control group (55.91 ng/L, 0.250 pg/ml respectively).

Conclusion: There were a positive correlation between specific anti *H. pylori* IgG level and IL-6 level in *H. pylori* associated with gastric ulcer diseases.

Introduction

Helicobacter pylori is considered to be one of the "gastric" helicobacters. In the stomach, it lives within or beneath the mucous layer adjacent to the epithelium. It is also found transiently in the duodenum, saliva, and feces. Infection with *H. pylori* may result in acute gastritis symptoms. Most infected patients develop into chronic active gastritis, which may lead to non-ulcer dyspepsia or duodenal ulcers. *Helicobacter pylori* has been associated with 90% of duodenal ulcers and nearly all gastric ulcers [1].

The key pathophysiological incident in *H. pylori* infection is the induction of an inflammatory response in the gastric mucosa, which is interceded and regulated by inflammatory cytokines manufactured by epithelial cells[2]. Polymorphisms in genes encoding cytokines such as interleukin (IL)-6, IL-8, and IL-10 influence the cytokines secretion levels and appear to contribute to the risk of developing gastroduodenal diseases[3,4,5]. Interleukin-6 is a pro inflammatory cytokine that functions as an inflammatory mediator and an endocrine regulator. In addition, it has an important

role in host defense mechanisms as a messenger between innate and adaptive systems [6].

The gastric mucosal levels of IL-6 are raised in *H. pylori* -associated gastritis and reduced after the eradication of the infection [2,7]. The aim of the present study was to identify the correlation between specific anti *H. pylori* IgG and IL-6 in *H. pylori* associated gastric ulcer diseases.

Materials and Methods

The study was conducted in Kirkuk province from 15th of December 2016 to 15th of June 2017, total of 90 patients were admitted to the Endoscopic Unit at "Gastroenterology and Hepatology in Azady Teaching Hospital/Kirkuk province" their age between 21-60 years. A total of 74 patients who did not administrated any treatment against *H. pylori* infection and 6 patients who administrated a treatment against *H. pylori* infection with 10 control group these were examined.

Using sterile equipments, a 6ml of blood was taken from each patient's vein after the diagnosis and confirmed type of gastrointestinal tract disorder by

gastroenterologist. The collected blood transferred directly into a plain tube special for the serum then it was put between (20-25)^oc for (15) minutes. After blood clotting, the serum was separated using a (2500)rpm centrifuge for (15) minutes.

Then, the serum was divided into two tubes each of them containing 3ml, the first tube was for the IL-6 examination by ELISA were manufactured by Korian biotech, China, UK, and the other tube was for the *H. pylori* IgG examination by ELISA were manufactured by Korian. biotech, China,UK.

Helicobacter pylori IgG ELISA

Reagent Preparation

1x Wash buffer is prepared by adding the contents of the bottom (25 ml,20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26^oc).

Assay procedure

Brought all specimens and kit reagents to room temperature (18-26^o c) and gently mix. The desired number of coated strips was placed into the holder. Negative control, positive control, and calibrator were ready to use. 1:21 dilution of test samples was prepared, by adding 10 ml of the sample to 200 ml of sample diluent. Mix well. A 100 ml of diluent sera was dispensed, calibrator and controls into the

appropriate wells. For the reagent blank, 100ml sample diluent was dispensed in 1A well position. The holder was tapped to remove air bubbles from the liquid and mixed well. Then incubated for 20 minutes at room temperature. Liquid from all wells was removed. Wells were washed three times with 300 ml of 1X wash buffer with blotting on absorbance paper. A 100 ml of enzyme conjugate was dispensed to each well and incubated for 20 minutes at room temperature. Enzyme conjugate was removed from all wells. Wells were washed three times with 300 ml of 1X wash buffer with blotting on absorbance paper. A 100 ml of TMB substrate was dispensed and incubate for 10 minutes at room temperature. A 100 ml of stop solution was added. O.D. was read at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Human Interleukin-6 ELISA kit

Reagent Preparation

All reagents should be brought to room temperature before use. All standards and samples should be run in duplicate. Dilution of standard solutions suggested are as follows:

320 ng/L	Standard No.5	120 ml Original Standard+120 ml Standard diluent			
160 ng/L	Standard No.4	120 ml Standard No5+120 ml Standard diluent			
80 ng/L	Standard No.3	120 ml Standard No.4+120 ml standard diluent 120ml Standard No4+ 120 ml Standard Diluent			
40 ng/L	Standard No.2	120 ml Standard No.3 + 120 ml Standard Diluent			
20 ng/L	Standard No.1	120 ml Standard No.2 +120 ml Standard Diluent			
Standard	S5	S4	S3	S2	S1
640 ng/L	320 ng/L	160 ng/L	80 ng/L	40 ng/L	20 ng/L

If the standard hasn't been run out, keep the remain at -20^oc. Diluted standard can't be reused. Dilute the wash buffer concentration (30x) with distiches water. The solution was discarded in the plate without touching the side. Then the plate was blot onto absorbent paper or other absorbent material. Each well was soaked with at least 0.35 ml wash buffer for 1-2minutes.

Assay Procedure

A 50 ml standard was added to standard well. Then a 40 ml sample was added to sample well and then a 10 ml anti-IL-6 antibody was added to sample wells, after that, a 50 ml streptavidin-HRP was added to sample wells and standard wells (Not blank control well). Mix well and cover the plate with sealer. Shake gently to mix them up. Incubate 60 minutes at 37^oc. The sealer was removed and the plate is washed 5 times with wash buffer. Wells was soaked in wash buffer for 30 seconds for 1 minute for each wash. For automated washing, all wells were aspirated and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto filter paper. A 50 ml substrate solution A was added to each well and then a 50 ml substrate solution B was added to each well. The plate was incubated and covered with a new sealer for 10 minutes at 37^oc at room temperature in the dark. A 50 ml stop solution was added to each

well. The blue color will change into yellow immediately. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 30 min after adding the stop solution.

Statistical Analysis:

Computerized statistically analysis was performed using Anova version 11 statistic program. Comparison was carried out using F-ratio. The P value > 0.05 was considered statistically significant, and for result which its P value was less than 0.01 was considered highly significant, while for those which its P value was greater than 0.05 considered non-significant statistically. Guidelines for interpreting correlation coefficient (r) :

- $0.7 < |r| \leq 1$ strong correlation
- $0.4 < |r| < 0.7$ moderate correlation
- $0.2 < |r| < 0.4$ weak correlation
- $0 \leq |r| < 0.2$ no correlation

Results

1. Relation of IL-6 Level with Anti *H. pylori* IgG level in the study groups

The results showed that the mean values of IL-6 level and anti *H. pylori* IgG level are showed a highly significant differences in the group of patients without treatment (225.592 ng/L, 1.338 pg/ml

respectively), while in the other group of patients who they administrated a treatment against *H. pylori* infection the mean values of IL-6 level and anti *H. pylori* IgG level showed a highly significant differences (102.60 ng/L 0.350 pg/ml respectively),

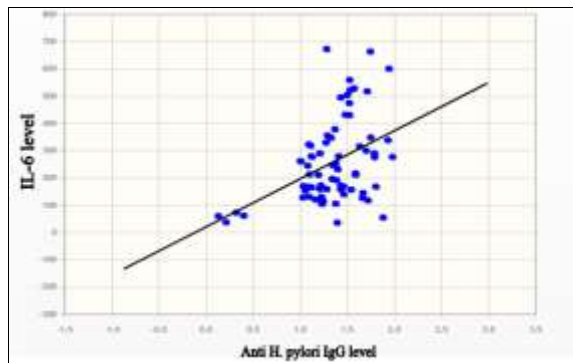
and in the control group the mean values of IL-6 level and the anti *H. pylori* IgG level were showed a highly significant differences (55,91ng/L, 0.250 pg/ml respectively) [Table 1].

Table 1: Relation of IL-6 Level with anti *H. pylori* IgG level

Study groups	No	Anti <i>H. pylori</i> IgG level pg/ml	IL-6 level ng/L	F ratio	P value	relation
		Mean \pm St.Dv	Mean \pm St.Dv			
Without treatment	74	1.338 \pm 0.358	255.592 \pm 150.850	14.498	0.0001	HS
After treatment	6	0.350 \pm 0.339	102.60 \pm 54.5342	4.592	0.001	HS
Control	10	0.250 \pm 0.267	55.91 \pm 27.8899	6.311	0.0001	HS

2. Correlation between anti *H. pylori* IgG level and IL-6 in patients without treatment.

The Correlation between anti *H. pylori* IgG level and IL-6 in patients without treatment were a moderate positive correlation between anti *H. pylori* IgG level and IL-6 in patients without treatment [fig. 1].

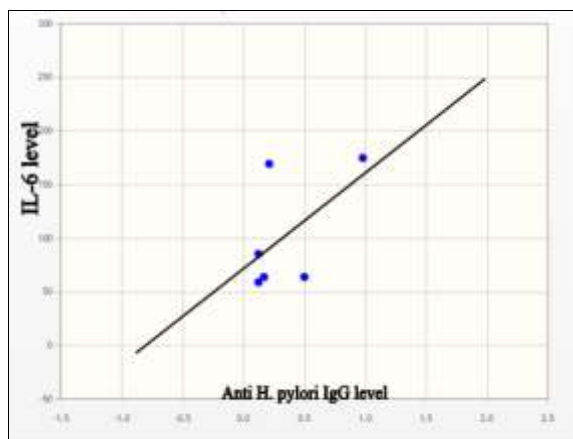


Correlation coefficient $r: 0.418$ ($0.4 < r < 0.7 =$ moderate positive correlation)

Figure 1 : Correlation between anti *H. pylori* IgG level and IL-6 in patients without treatment

3. Correlation between anti *H. pylori* IgG level and IL-6 in treated groups.

There were a moderate positive correlation between anti *H. pylori* IgG level and IL-6 in treated patients [Fig. 2].



Correlation coefficient $r: 0.554$ ($0.4 < r < 0.7 =$ moderate positive correlation)

Figure 2: Correlation between anti *H. pylori* IgG level and IL-6 in treated group

Discussion

Interleukin-6 is a pro inflammatory cytokine that functions as an inflammatory mediator and an endocrine controller. In addition, it has an important role in host defense mechanisms as a messenger between innate and adaptive systems [6].

The gastric mucosal levels of IL-6 are raised in *H. pylori*-associated gastritis and reduced after the eradication of the infection [2,6].

1. Relation of IL-6 level with serum anti *H. pylori* IgG level in the study groups

In the present study showed that the mean values of IL-6 level and anti *H. pylori* IgG level are showed a highly significant differences in the group of patients without treatment (1.338 ng/L, 225.592 pg/ml respectively), while in the other group of patients who they administrated a treatment against *H. pylori* infection the mean values of IL-6 level and anti *H. pylori* IgG level showed a highly significant differences (0.350 ng/L, 102.60 pg/ml respectively), and in the control group the mean values of IL-6 level and the anti *H. pylori* IgG level were showed a highly significant differences (55,91ng/L, 0.250pg/ml respectively).

The present study agreed with [8].who showed high level of IL-6 related to *H. pylori* IgG positive, with normal level of interleukin-6 and *H. pylori* IgG in control groups, other studies also covenant with the present study [2]. who revealed an increase interleukin - 6 level in *H. pylori* associated gastritis and dramatically decrease after eradication of infection [9].who also showed that circulatory IL-6 level were significantly higher in *H. pylori* infected patients as compared to *H. pylori* negative patients.

A strong immune response to *H. pylori* enhanced the systemic inflammation, which was reflected in an increased level of serum IL-6 [9]. *H. pylori* is the major recognized etiological agent inducing gastric inflammatory responses [10]. These responses can be considered to have two components: an acute inflammatory response characterized by intraepithelial and interstitial neutrophilic infiltration and chronic inflammatory responses associated with increased numbers of mononuclear cells in the lamina propria, including lymphocytes, monocytes/macrophages, and plasma cells [11]. These two responses may be regulated differentially following

induction of cytokines involved in the inflammatory cascade, including tumor necrosis factor alpha (TNF- α), IL-1, IL-6, and IL-8 [12]. IL-6 may be involved in chronic inflammatory changes through its broad effects on growth and differentiation of mononuclear cells, including T and B lymphocytes and macrophages [13].

2. Correlation between anti *H. pylori* IgG level and IL-6 in patients without treatment

The current study showed that there were a moderate positive correlation between anti *H. pylori* IgG level and IL-6 in patients who they did not administrated any treatment against *H. pylori* infection.

The present study were in agreement with [10,14] who they showed that *H. pylori* might induce significant increases in serum IL-6 levels and there were a positive correlation between them, the current study also were in agreement with [11,15] who they revealed *H. pylori* infection might increase the expression of IL-6 means there were a moderate positive correlation between them, [12,16] also revealed that *H. pylori* induces pro inflammatory cytokines, such as IL-6 signaling pathways, [13,17]. who they revealed that *H. pylori* infection triggers the release of inflammatory cytokines such as IL-6, [14,18]. who they showed that the presence of *H. pylori* caused a significant increase in release of IL-6 the result showed that statistically there were a positive correlation between them.

The description of this result were the process of *H. pylori* infection, the activation of the immune system can lead to the recruitment of a wide variety of inflammatory cells and mediators, and the activation of nuclear factor- κ B (NF- κ B) and pro- and anti-inflammatory cytokines [15,19].

3. Correlation between anti *H. pylori* IgG level and IL-6 in treated groups

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The current study revealed that there were a moderate positive correlation between anti *H. pylori* IgG level and IL-6 in patients who they previously treated against *H. pylori* infection.

The present study were in agreement with [16,20]. *H. pylori* eradication has been shown to reduce the levels of pro-inflammatory cytokines in which there were a positive correlation between them, [17,21]. who they showed that the levels of IL-6 were low in *H. pylori* -positive patients who they treated against *H. pylori* infection, [18,22]. who they showed that the level of IL-6 were decreased in patients who treated against *H. pylori* infection and there were a positive correlation between them.

The description of that result were this bacteria is capable to colonize and persist in the mucus layers of the human stomach. Infection affects almost half of the world's population and it is responsible for the most frequent and persistent infection of the gastrointestinal tract universal [23].

The key pathophysiological incident in *H. pylori* infection is the induction of an inflammatory response in the gastric mucosa, which is interceded and regulated by inflammatory cytokines manufactured by epithelial cells [11]. Polymorphisms in genes encoding cytokines such as interleukin (IL)-6, IL-8, and IL-10 influence the cytokines' secretion levels and appear to contribute to the risk of developing gastro duodenal diseases [24].

Conclusions

1. Level of IL-6 were higher than controls in patients with gastritis, duodenal ulcer, gastric ulcer and duodenitis.
2. There were a positive correlation between specific anti *H. pylori* IgG level and IL-6 level in patients with and without treatment.

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تقييم العلاقة بين الاجسام المضادة للبكتريا الملثوية البوابية والانترلوكين-6 لدى المصابين بالتهاب المعدة في محافظة كركوك

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الملخص

تمهيد: تعتبر البكتريا الملثوية البوابية السبب الأكثر شيوعا لالتهاب المعدة المزمن، وأيضاً في مرض القرحة الهضمية، وان استمرارية بقائها في طبقات المخاط للمعدة يسبب ارتفاع مستوى السيستوكينات المؤيدة للالتهابات مثل إنترلوكين-6.

المواد وطرائق العمل: أجريت الدراسة في مدينة كركوك في الفترة من 15 ديسمبر 2016 إلى 15 يونيو 2017 في مستشفى أزاوي التعليمي في محافظة كركوك، وتم اجراء فحص التلازن المناعي الانزيمي ELISA للتحري عن مستوى الاجسام المضادة للبكتريا الملثوية البوابية ومستوى الانترلوكين-6.

النتائج: اظهرت النتائج ان من مجموع 90 مريض كان مستوى انترلوكين 6- ومستوى الاجسام المضادة للبكتريا الملثوية البوابية في مجموعة من المرضى غير المعالجين ضد عدوى الملوية البوابية (225.592 نانوغرام / لتر، 1.338 بيكوغرام / مل على التوالي) وفي مجموعة من المرضى (102.60 نانوغرام / لتر، 0.350 بيكوغرام / مل على التوالي) أعلى بكثير مقارنة مع مجموعة السيطرة (55.91 نانوجرام / لتر، 0.250 بيكوغرام / مل على التوالي).

الاستنتاج: بينت النتائج ان هناك علاقة ارتباط إيجابية بين مستوى الاجسام المضادة للبكتريا الملثوية البوابية و مستوى إنترلوكين-6 في المرضى المصابين بالبكتريا الملثوية البوابية المرتبطة بأمراض قرحة المعدة.

الكلمات: البكتريا الملثوية البوابية، التهاب المعدة، مرض القرحة الهضمية.