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IMMUNO-PATHOGENESIS OF *HELICOBACTER PYLORI* AND DIABETES MELLITUS TYPE II

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Abstract

Helicobacter pylori is one of the most common human pathogens that can cause gastrointestinal (GI) disorders, including simple gastritis, gastric ulcer and malignant gastritis. In some cases, such as immunodeficiency and underlying diseases, it can be problematic as opportunistic infections. Diabetes mellitus (type 2) (DMT2) is one of the *H. pylori* underlying diseases. Results of current study showed a high incidence of infection in the age group (45-55) years old and (54-65) years old as (no. = 23.0, 24.0) and percentage as (38.3, 40)% respectively. The study showed high positive rate of anti-*Helicobacter pylori* IgG and IgM antibodies in patients group compared to healthy persons, as the positivity of antibody IgG was 90%, while the antibody IgM was 73.3% compared to the control group. The results also showed a high IL-6 level in patients 94.45 ng/L compared with healthy persons 63.28 ng / L with a difference Significantly ($P \leq 0.01$) and low level of complement proteins C4 and C3 in patient 527.50 µg/ml and 0.27 mg/ml, respectively, with a significant difference (groups $P \leq 0.05$) compared with healthy persons. The results also showed increase of acute phase protein (CRP) positivity in the patients group which reached (18) by 30%.

Key words*:* anti-*H.pylori* IgG, IgM, Diabetes mellitus type II, IL-6,C3,C4 and CRP.

Introduction

Helicobacter pylori are an spiral-shaped gram-negative pathogenic bacterium that specifically colonizes in the gastric epithelium and causes several acute and chronic gastro-duodenal diseases including chronic gastritis, peptic ulcer disease and/or gastric malignancies (Testerman & Morris, 2014). It infects 50% of the world's people, although most infected individuals have no clinical symptoms (Krzyzek & Gosciniak, 2018). Due to its linkage to local and systemic gastric inflammation, it has also been linked to other resulting corresponding conditions such as cardiovascular disease, unexplained iron deficiency anemia, nonalcoholic fatty liver disease, Diabetes mellitus type 2 and insulin resistance (Dogan *et al*., 2015).

Diabetes mellitus type 2 is a multifactorial, chronic disease and occurs due to a mixed of hazard factors are environmental and genetic factors. DMT2 is the most common form of diabetes, responsible for (90%-95%) of people of all types of diabetes (Cho *et al.,* 2018). This the type occur and develops when the body fails to use the insulin due to insulin resistance (IR) with gradual loss to ability betacells to make enough insulin (Karuna, *et. al.,* 2013). Although *H. pylori* infection and diabetes mellitus are two separate diseases, it has been observed that poor glycemic control in DMT2 is related to higher rates of *H. pylori* infection. *H. pylori* infection has been described as one of the most common complications in diabetics with gastric symptoms (Devrajani *et al.,* 2010). Trend of DMT2 is changed from metabolic disorder to inflammation as effects of the pro and anti-inflammatory cytokines like interleukin-6 (IL-6) and C-reactive protein (CRP) has been reported in insulin signaling pathways, cross-linking and ultimately developing insulin resistance in β-cells of pancreas which further risks to DMT2 (Hameed *et al*., 2015).

The complement system was defined as a heat-labile substance that assisted or killing of bacteria by heat-stable antibodies in the blood. Over time, it has come to be recognized as a group of proteins functioning as a humoral immune amplification system in innate immunity as well

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as a regulator of the adaptive immune response. Besides playing an integral role in host defense against infection, the major functions of the complement system include acting as an interface between innate and adaptive immunity and clearing immune complexes and apoptotic cells. Complement proteins activation occurs in the plasma and extracellular space (Carroll & Isenman, 2012). The complement system is mainly associated with innate immunity in addition to be involved in metabolic events. Certainly, complement components C3, C4 are associated with diabetes mellitus, cardiovascular risk and the metabolic syndrome. (Jiffri *et al*., 2018). C-reactive protein (CRP) is an acute-phase reactant produced primarily in the liver under the stimulation of adipocyte-derived proinflammatory cytokines, including IL-6. CRP is the most commonly measured circulating marker for subclinical inflammation, with widely available, stable and standardised assays for its measurement (Pearson *et al.,* 2003), this mediator increased in response to infection and inflammation (Fronczyk *et al*., 2014). The aim of current study was designed to evaluate the serum level of immunoglobulin (IgG, IgM) for bacteria, Levels of complement proteins (C3, C4), the level of interleukin 6 (IL-6) and acute phase protein (CRP) among diabetes mellitus infected by *H pylori*.

Materials and Methods

Study Samples

This study included 88 participants, 28 healthy persons as a control group and 60 patients with *H. pylori* and DMT2 (30 males and 30 females) with ages group from ranged from 35 to 80 years. The patients were selected from advisory clinic in Baquba Teaching Hospital in Baquba city during the period from the beginning of October 2019 to the end of January 2020. All information about patients recorded in questioner.

Blood Sampling

Five milliliters of venous blood was drawn by disposable syringe. Placed in a sterile plane tube and allowed to clot; then, Serum was separated from the cells by centrifugation and then divided into small portions and kept frozen at "20°C until analysis. These sera were used for estimating IL-6, anti-*H. pylori* IgG, IgM,Complement protein C3, C4 and CRP.

Methods

1-Anti-*h.pylori*IgG, IgM, were measured by sandwich enzyme linked immune sorbent assay kit which provided from demeditec company, Germany and IL-6, C3, C4 from Shanghai Company, china.

2-CRP was detected by C - reactive protein Test Kit by Latex Agglutination from Spainreact Company, Spain.

Principle of the assay

1-The microtiter plate provided in this kit has been pre-coated with specific antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then added Horseradish Peroxidase (HRP)-conjugated -anti-human immunoglobulin to each well and incubate. Finally, substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. Calculate the concentrations of samples (Dhar *et al*., 1998).

2- This test is based on the principle of the immunological reaction that occurs between the human acute phase protein (CRP) and the corresponding antibody to this antigen bound to the latex molecules (Abrams & Jonathan 2005).

Statistical analysis

Data were processed and analyzed using the Statistical Package of the Social Science (SPSS 20). Descriptive formula variables were described in number and percentage formula and compared using Chi-square test. All results were expressed as mean ± standard error deviation. Quantitative variables were expressed as (mean ± SED) and compared using student t-test. The linear relationship between variables was assessed by Pearson's correlation coefficient (r). $P < 0.05$ was considered statistically significant) Levesque, 2007).

Results and Discussion

Since the discovery of Helicobacter pylori by Barry J. Marshall and Robin Warren in 1984, where they found that the bacteria was present in almost all subjects with active chronic gastritis, duodenal ulcer, or gastric ulcerand concluded that it may be an important etiological factor of these diseases, since then and the bulk of research was focused on the effect of the bacteria on the gastro-intestinal region. Current data suggest an extra-gastric role of H. pylori, there is evidence that H. pylori infection is associated with increased incidence of DMT2 (Joen, 2012).

The chi-square ($X2$) test did not show a significant difference in age between the two groups (p=0.233**)** table 1. The results of this study showed that the highest percentage of infection was recorded in the age groups (45-54) and (55-64) years, The prevalence of the metabolic syndrome rose with age reaching peak levels in the sixth decade and fifth decade is paralleled by similar increases in the prevalence of overweight and obesity

Table 1: Distribution of the study groups according to the percentages of age groups.

Age groups (years)	Patients N (%)	Controls N (%)	P-value
(35-44)	5(8.3%)	7(25%)	
(45-54)	23(8.3%)	7(25%)	
(55-64)	24(8.3%)	11(39.3%)	0.233 NS
(65-44)	7(8.3%)	2(7.1%)	
(75-80)	1(8.3%)	1(3.6%)	
Total	60	28	

NS: No significant difference ($P > 0.05$).

(Park *et al*., 2003), key related factors in the development of visceral adiposity, insulin resistance, dyslipidemias, high blood pressure and impaired glucose metabolism. In addition, aging per se is associated with evolution of insulin resistance, other hormonal alterations and increases in visceral adipose tissue, (Akram, 2013) all of which are important in the pathogenesis of the DMT2. Documented results were consistent with the findings of other authors (Jasim, 2015).

Table 2 The chi-square (*X2*) test did show high significant difference of anti-*H. pylori* IgG, IgM in patient groups compared with controls and no significant difference of CRP in patient compared with healthy groups. The study aims to determine the prevalence of *H. pylori* infections in DMT2 and non-diabetic patients and to identify the association between H. pylori infections and diabetes mellitus.. Its similar results were also detected in studies conducted with (Tawfeeq *et al*., 2019; Ebule *et al*., 2017) and disaggregation with (Jafarzaden *et al*., 2013). The results noted that CRP is significantly greater in the patients compared with a healthy group. These results are compatible with those found by (Thejaswini *et al*. 2013), who described a higher C-reactive protein in patients. Since, CRP is considered to be a sensitive indicator of inflammation, a higher CRP level in infection may suggest that inflammation could be involved in the pathogenesis of diabetes and early atherosclerotic processes (Nagwa *et al.,* 2016), also, raised levels of C- reactive protein is related to the elevated with insulin resistance (XU *et al*., 2008).

Table 3 The study showed that the proportion of males of anti-*H. pylori* IgG, IgM is more than that of females, without significant difference while it show the CRP in female is more than male. Also the study did not show any significant difference among patients depending on age groups.

Table 4 shows the serum levels of some immunological parameters in two studied groups. The IL-6 level show a high significant increase ($P < 0.01$) for patients (94.45±4.21) ng/l compared to control (63.28±7.17) ng/l .levels of C3showed significant decrease ($P \leq 0.05$) for patients (527.50±30.10)) µg/ml) as compared to the controls, (735.52±194.88) µg/ml andlevels of serum C4showed a high significantly decrease ($P < 0.01$) in patients (0.27±0.01) mg/ml compared to control (0.37±0.06) mg/ml.

Recorded results revealed that the IL-6 is increased significantly ($P < 0.01$) in patients groups when compared with the controls.Interleukin-6 (IL-6) is a multifunction cytokine that regulates immune response, acute phase

Table 2: Comparing immunological parameters with the two study groups.

Para-meters	lgG N (seropositivily)	IgMN (seropositivily)	CRPN (seropositivily)
Patients	54(90.0%)	44(73.3%)	18(30%)
Controls	17(60.7%)	0(0.0%)	7(25%)
p-value	0.001*	0.000**	0.052NS

NS: No significant difference ($P > 0.05$)
*Significant difference ($P \leq 0.05$)
**Significant difference (P value < 0.01)

Table 3: Comparing immunological parameters with gender and age.

Para-meters	lgG N (seropositivily)	IgMN (seropositivily)	CRPN (seropositivily)
Male	28 (93.3%)	23(76.6%)	7(23.3%)
Female	26 (80%)	21 (70%)	11(36.6%)
P value	0.421 NS	0.672 NS	0.031*
Age group (years)	IgGN (seropos -itivily)	IgMN (seropos -itivily)	CRP N (seropos -itivily)
35-44	5(8.3%)	5(8.3%)	2(3.3%)
45-54	23(38.3%)	17(28.3%)	6(10%)
55-64	24(40%)	15(25%)	9(15%)
65-74	8(13.3%)	6(10%)	1(1.6%)
P value	0.435NS	0.314 NS	0.536 NS

NS: No significant difference ($P > 0.05$).
*Significant difference ($P \leq 0.05$).

Table 4: Comparing some immunological parameters with the two study groups.

Parameters	Group	Mean± SEM	P-value
Serum IL-6	Patients	94.45±4.21	**0.000
	Control	63.28±7.17	
C 3	Patients	527.50±30.10	0.003*
	Control	735.52±194.88	
C 4	Patients	0.27±0.01	0.000**
	Control	0.37±0.06	

*Significant difference ($P \leq 0.05$)
**Significant difference ($P < 0.01$).

reactions and hematopoiesis and may play a central role in host defense mechanisms and it's one of several pro inflammation cytokines that have been associated with insulin resistance (Ibfelt *et al*., 2014). These results agreed with several studies (Bashir *et al*., 2020; Majeed & Marbut, 2019). The study also show C3, C4 are decreased significantly in patient persons compared with healthy groups. The results agree with (Saleh, 2011; Zhang *et al.,* 2012) and disagree with (Mustafa *et al*., 2019). Waldport (2001) referred to under physiological conditions; complement promotes the clearance of immune complexes, an important way of eliminating antibody-coated bacteria. If, however, immune complexes cannot be eliminated, complement becomes chronically activated leading to increased consumption of the components. This might be the reason for lower level of complement C3 that could affect the formation of membrane–attack complex and lower bactericidal activity.

Table 6: Correlation coefficient of immunological parameters in patients group.

		IL-6	C3	C4	IgG	IgM	CRP
IL-6	R	1	.748**	.825**	.255*	.220*	.072
	P		.000	.000	.016	.036	.507
C3	R	.748**	1	.831**	-.107	-.142	.150
	P	.000		.000	.319	.188	.163
C4	R	.825**	.831**	1	-.132	-.101	.171
	P	.000	.000		.221	.339	.112
lgG	R	.225*	-.107	-.132	1	.204	.068
	P	.016	.319	.221		.057	.527
lgM	R	.220*	-.142	-.103	.103	1	.041
	P	.036	.188	.339	.341		.701
CRP	R	.072	.150	.171	.068	.041	1
	P	.507	.163	.112	.527	.701	

R=Pearson Correlation, P=Probability,*=positive correlation.

Table 5: The relationship between some immunological parameters with the gender of patients and age groups.

Para-meters	IL-6 Mean± SEM	C3 Mean± SEM	C4 Mean± SEM
Male	98.82±4.59	634.14±36.10	0.30±0.01
Female	90.82±7.05	420.86±40.00	0.23±0.02
P value	0.138 NS	0.063NS	0.099NS
Age group (years)	IL-6 Mean± SEM	C3 Mean± SEM	C4 Mean± SEM
35-44	110.42±20.40	512.92±110.84	0.40±0.09
45-54	90.98±7.25	506.64±50.29	0.22±0.02
55-64	97.77±6.06	555.38±46.10	0.28±0.02
65-74	84.47±9.35	512.94±92.45	0.26±0.04
P value	0.116NS	0.686NS	0.308NS

NS: no significant difference (P > 0.05).

Table 5 revealed that IL-6, C3 and C4 levels did not show any significant difference among patient groups depending on gender and age groups.

The present study showed no significant difference (P>0.05) in the level of all immune parameters(IgG, IgM, IL-6, C3 and C4) table 3, 5 with gender and age groups patients, except CRP show significant difference($P \leq 0.05$) in gender table 3. The lack of a statistically significant difference in level of immune parameters with gender and age groups may be due to the gender-based immune defense mechanisms, since males and females show the same immune cells to the immune response that occur in the patient's body, which may be somewhat similar regardless of the As the interaction within the patient's body leads to the activation of immune cells responsible for immunologic response in the serum of patients with diabetes (Voskuhl, 2011). Table 6 Pearson correlation analysis revealed a significant positive and no significant correlation between immunological parameters as shown in table 6.

Conclusions

During the current study, high significant increase of anti-*H.pylori*IgG, IgM, IL-6 in patient groups compared with controls and significant decrease of C3, C4 in patient compared with healthy groups. Patient shows no significance different between immunological Parameters with the gender of patients and age groups. Also showed positive correlation between some immunological Parameters

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