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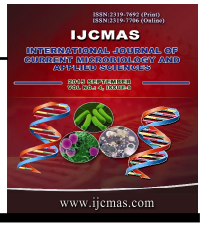

Original Research Article

Study of Some Immunological Aspects of *Helicobacter pylori* Iraqi patients with type 2 Diabetes mellitus

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Keywords

TNF-α, *Helicobacter pylori*, Diabetes mellitus, IL-6

A B S T R A C T

Aim of the study is to investigate the association of the cytokines (tumor necrosis factor alpha TNF-α and interleukin 6 IL-6) levels a major inflammatory mediators, with metabolic parameters like glycolated hemoglobin and glucose in Iraqi patients with type 2 diabetes mellitus with *H.Pylori* infection. TNF-α and IL-6 concentrations were measured by enzyme-linked immunosorbent assay (ELISA), while Hba1c and glucose were measured by spectrophotometer technique. Patients group was divided to subgroups according to that the infection with *Helicobacter pylori* which are + ve *H. pylori* group and -ve *H. pylori* group**.** sixty one diabetic patients were compared with (31) healthy subjects to assess the studied parameter. TNF-α was found to be significantly elevated in diabetic subjects versus the control group, also there was a significant difference in TNF-α level in T2DM *H. pylori* +ve versus T2DM *H. pylori* -ve group. IL-6 was found to be high in T2DM group when compared to healthy subjects and there was a significant difference in IL-6 level between control and T2DM *H. pylori* +ve patients groups. Compared to healthy groups, IL-6 and TNF-α levels were found to be substantially higher in patients with type 2 diabetes mellitus.

Introduction

Diabetes is a set of metabolic disorders characterized by high level of blood sugar due to deficient insulin secretion, action, or both (ADA, 2014). Type 2 Diabetes Mellitus (T2DM) also known as non-insulin-dependent diabetes mellitus or adult-onset diabetes. It is the result of insulin resistance and relative insulin deficiency that occurs due to decrease insulin production from the pancreas (Cong H, 2014). Patients with this form of diabetes are at high risk of developing macro and microvascular complications (El Hadidy M,. 2009). Initial data identify a strong relation between infection by H. pylori and the accurance of metabolic syndrome and T2DM. *H. pylori* infection is known to participate in the development of insulin

resistance result from chronic inflammation with glucose and lipids abnormalities that leads to growing awareness of its role in Type2 DM(Lou Rose Malamug, 2014).

It's frequently thought that the chronic inflammation caused by H. pylori colonization in the gastric epithelium intensely related to the T2DM pathogenesis. It associates with a non specific initiation of the innate immune system. Also, It heightens the expression of cytokines such as IL-6, CRP, and IL-1β, as well as tumor necrosis factor (TNF-α) that affect many tissues cause recognizable features of T2DM .Stimulate of TNF-α production in the adipose tissue may be a critical role by which fat cells cause peripheral IR . Indirectly: stimulate free fatty acid oxidation. Stimulation of cytokines (e.g., IL-6 and CRP) or insulin counter-regulatory hormones and impair of endothelial function.

Directly: alter rule on glucose carrier protein GLUT4, insulin receptor substrates, or glucose-stimulated insulin discharge by pancreatic β-cells and the development of diabetes (GRUYS E, 2005). High levels of IL-6 predict the ocurance of T2DM and further ensure a likely effect for inflammation in diabetogenic.IL-6 is thought to modify body weight, lipid metabolism and adipocyte glucose (Cong H, 2014).The stimulation of interleukin 6 (IL-6) and TNF α in the liver lead to the production of C-reactive protein (CRP) and in adipocytes by TNF α and resistin. CRP increases the production of intercellular adhesion molecule 1 (ICAM-1) and monocyte chemoattractive protein-1 (MCP-1) by the endothelial cells (Denise M, 2004).Moderate elevation in CRP levels have been shown to be a significant predictor of the risk of DM directly by a variety of mechanisms (Michael J, 2008).

Material and Methods

The study was conducted on (61) type 2 diabetic patients(patient group) (male/female =29/32), an average age of 45 years, BMI <40 kg/m2 randomly selected from those attending the National Diabetes Center for Treatment and Research at Al-Mustansiriya University, Baghdad/Iraq. between September 2014 - April 2015. Informed consent was obtained from each subject. Patients with concurrent acute illnesses, malignancy, and active immunological diseases; medical history of clinical cardiovascular disease; medical history included the diseases (hypertension, rheumatoid arthritis, anemia, bronchial asthma) or medications (warfarin, acetylsalicylic acid, alpha-methyldopa, vitamins, tramadol,simvastatin) that interfered with HbA1c % measurements, and smoking history were excluded from the study. The *H.pylori* infaction were detected by using enzyme linked immunosorbent assay anti-H. pylori IgG (DRG, USA) and and anti- Cag A IgG antibodies(DRG,USA). Serum TNF-α was determine by using ELISA technique (Phoenix Pharmaceuticals, USA) also IL-6 determine by it (RayBiotech, USA). The plasma glucose was measured by using kit from Randox Laboratories and Hba1c determined by using spectrophotometer kit (Human Company, Germany).

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences-version 22). The significance of difference of different means (quantitative data) were tested using Students-t-test or ANOVA test. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ2-test) with

application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

Results and Discussion

The prevalence of anti-*H. pylori* IgG antibodies in diabetic patients (89.94±63.38 NTU/ml) was significantly higher than that observed in healthy subjects (5.60±2.73NTU/ml).The mean titer of anti-*H. pylori* CagA IgG antibodies for patients (118.12±93.39 DU/ml) was significantly higher than that observed in healthy control group (10.14±4.63 DU/ml).

Comparisons of +ve and -ve *H. pylori* T2DM patients number according to IgG and IgG Cag A of *H. pylori*

In T2DM group, the observed number 47 (77%) were + ve H. pylori Cag A patients and14(23%) were -ve *H. pylori* patients.

As compared to the mean level TNF- α (29.309±2.220 pg /ml) for control group, significant elevation (32.190±8.136 pg /ml) in the mean serum level of TNF-α for T2DM patients group, A significant increase (31.845±7.415pg /ml) in TNF- α mean level was found in T2DM *H. pylori* +ve patients groups and a non significant increase (33.346±10.446pg /ml) was found in T2DM *H. pylori* -ve patients groups as compared to T2DM *H. pylori* +ve patients and control groups. But, The difference in the mean level was a non significant p= 0.549 between +ve and –ve *H. pylori* T2DM patients.

As compared to the mean level IL-6 (4.138±2.276 pg /ml) for control group, significant elevation (7.525±10.805pg /ml) in the mean serum level of IL-6 for T2DM patients group, A significant increase (6.504±7.374 pg /ml) in IL-6 mean level was found in T2DM H. pylori +ve patients groups and a non significant increase (10.950±18.168 pg /ml) was found in T2DM H. pylori -ve patients groups as compared to T2DM H. pylori +ve patients and control groups. Also, The difference in the mean level was a non significant p = 0.386 between +ve and –ve H. pylori T2DM patients.

Comparisons of IL-6 between +ve and -ve *H.pylori* T2DM patients

There was no correlation between IgG level with TNF- alpha and with IL6 (r =-0.047) (p=0.755) and (r =0.002) (p=0.989) respectively.

Correlation of the levels of IgG in T2DM +ve *H.pylori* patients with TNF- alpha and IL-6

There was no correlation between IgG Cag A level with TNF- alpha and with IL-6 (r =0.044) (p=0.767) and (r =0.155) (p=0.297) respectively.

Correlation of the levels of IgG Cag A in T2DM +ve *H.pylori* patients with TNF-alpha and IL-6

There were a weak direct significant correlation (r =0.363) (p=0.012) between FGT and IgG level and no correlation between IgG and RGT and HbA1c in +ve *H.pylori* T2DM pateins.

Correlation of the levels of IgG level in T2DM +ve *H.* pylori patients with FGT, RGT and HbA1c

The present study show indirect significant correlation (r =-0.339) (p=0.020) and(r =-0.351) (p=0.016) between FGT and HbA1c

and IgG level respectively and no correlation between IgG and RGT in +ve *H. pylori* T2DM patients.

Correlation of the levels of IgG Cag A (DU/ml) level in T2DM +ve *H.* pylori patients with FGT, RGT and HbA1c

To discriminate between T2DM patients and controls by employing the forthcoming investigated parameters, the ROC analysis was applied. Such analysis permits to organize the parameters according to the ROC area that can occupy and if such occupation is significant or not. The ROC analysis revealed the descending order (IL-6 = 0.577; TNF-alpha= 0.564) of parameters that showed a significant variations (Figure 3)

The specificity and sensitivity of TNF-alph and IL-6 level measuring was compared between T2DM *H. pylori* -ve patients and control groups and found that the (area under the curve = 0.763) (area under the curve = 0.541) for TNF-alpha and IL-6 respectively.

Table.1 Comparisons of +ve and -ve H. pylori T2DM patients number according to IgG and IgG Cag A of *H. pylori*

***H. pylori* seropositivity**	**Range**	**T2DM *H. pylori* +ve**		**T2DM *H. pylori* -ve**		**P value**		
		No	**%**	**No**	**%**	**HP+ xC**	**HP-xC**	**PH+ xPH-**
H. pylori	Positive	47	77.0	-	-			
	Negative	14	23.0	-	-			
IgG (NTU/ml)	Positive (>20)	47	100	1	7.1	-	-	-
	Negative	-	-	13	92.9			
	Mean±SD (Range)	110.2±52.08 (33.0-196.49)		21.99±49.90 (2.25-194.72)		0.0001#	0.241	0.0001#
IgG Cag A (DU/ml)	Positive(>18)	47	100	1	7.1	-	-	-
	Negative	-	-	13	92.9			
	Mean±SD (Range)	149.5±83.44 (32.4-320.2)		12.77±10.28 (5.9-47.38)		0.0001#	0.240	0.0001#
#Significant difference using Students-t-test for difference between two independent means at 0.05 level								

Table.2 Correlation of the levels of IgG in T2DM +ve *H.pylori* patients with TNF- alpha and IL-6

T2DM		**IgG (NTU/ml)**	
		T2DM *H pylori* +ve	**T2DM *H pylori* -ve**
TNF-alpha (pg/ml)	r	-0.047	0.067
	P	0.755	0.819
IL-6 (pg/ml)	r	0.002	-0.133
	P	0.989	0.650
*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.			

Table.3 Correlation of the levels of IgG Cag A in T2DM +ve *H.pylori* patients with TNF- alpha and IL-6

T2DM		**IgG Cag A (DU/ml)**	
		T2DM *H pylori* +ve	**T2DM *H pylori* -ve**
TNF-alpha (pg/ml)	r	0.044	0.000
	P	0.767	0.999
IL-6 (pg/ml)	r	0.155	0.599*
	P	0.297	0.024
*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.			

Table.4 Correlation of the levels of IgG level in T2DM +ve H. pylori patients with FGT, RGT and HbA1c

T2DM		**IgG (NTU/ml)**	
		T2DM *H pylori* +ve	**T2DM *H pylori* -ve**
FGT (mg/dl)	r	0.363*	0.404
	P	0.012	0.152
RGT (mg/dl)	r	0.086	0.106
	P	0.567	0.718
HbA1C (%)	r	0.064	0.493
	P	0.668	0.073
*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.			

Table.5 Correlation of the levels of IgG Cag A (DU/ml) level in T2DM +ve H. pylori patients with FGT, RGT and HbA1c

T2DM		**IgG Cag A (DU/ml)**	
		T2DM *H pylori* +ve	**T2DM *H pylori* -ve**
FGT (mg/dl)	r	-0.339*	0.112
	P	0.020	0.703
RGT (mg/dl)	r	-0.191	0.119
	P	0.199	0.686
HbA1C (%)	r	-0.351*	-0.019
	P	0.016	0.949
*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level.			

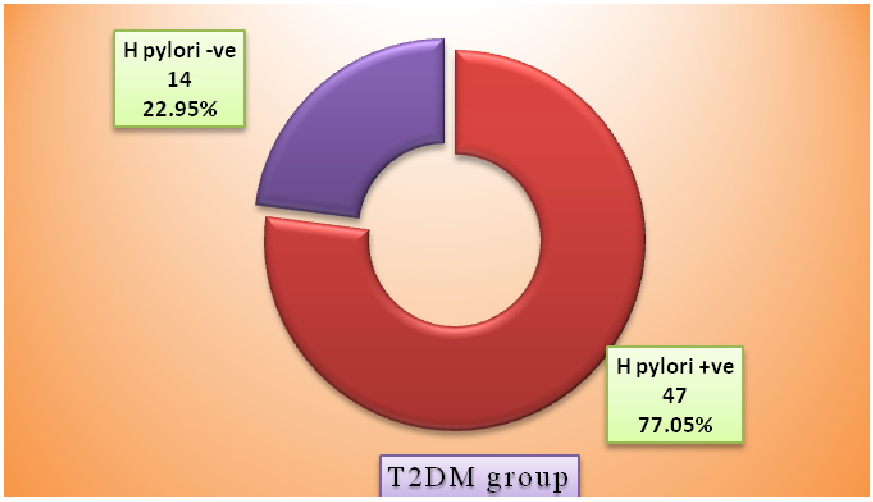


Figure.1 Comparisons of TNF-alpha level between +ve and -ve H. pylori T2DM patients.

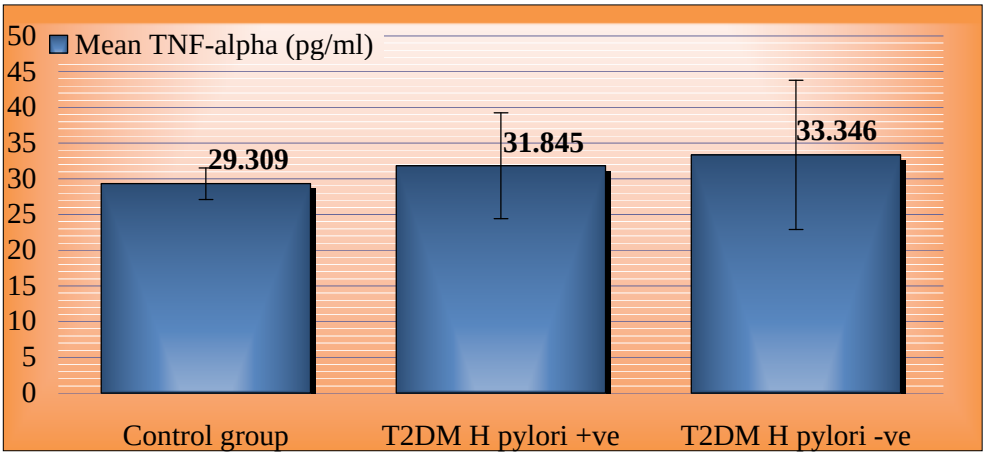


Figure.2 Comparisons of IL-6 between +ve and -ve *H.pylori* T2DM patients

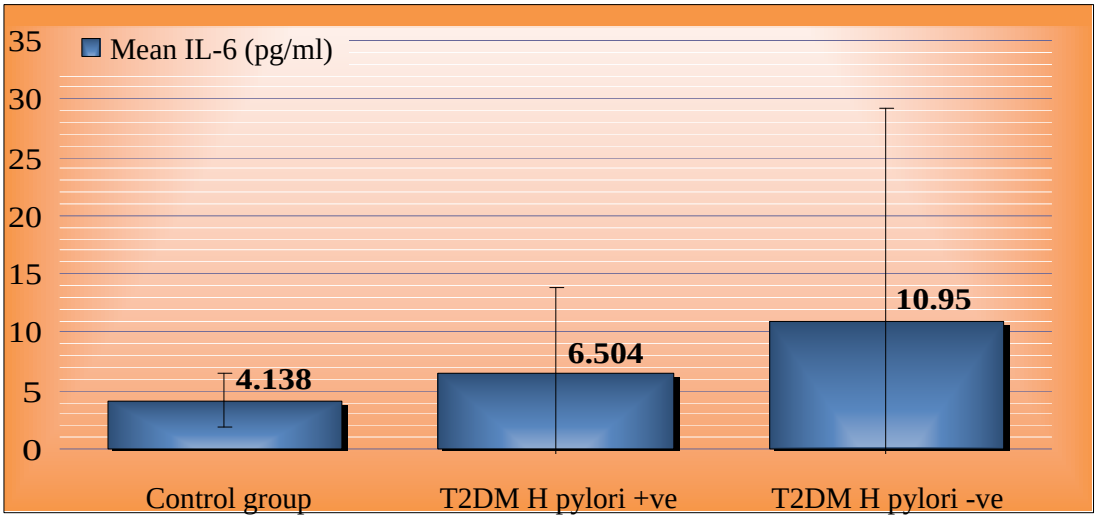


Figure.3 Receiver operator curve (ROC) analysis for the investigated parameters in T2DM +ve *H.pylori* patients and controls

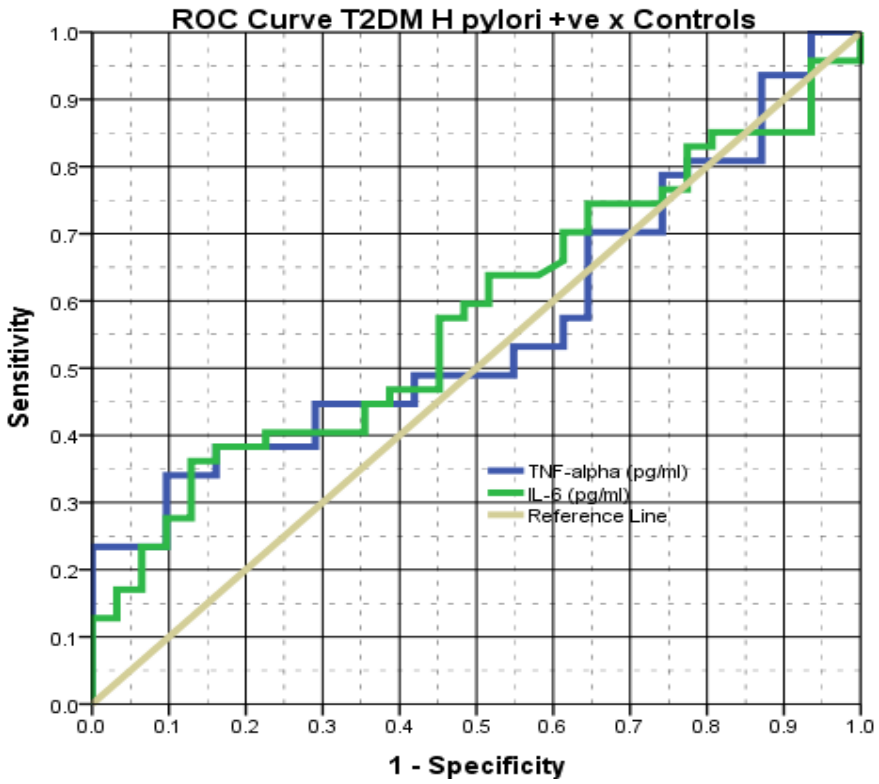
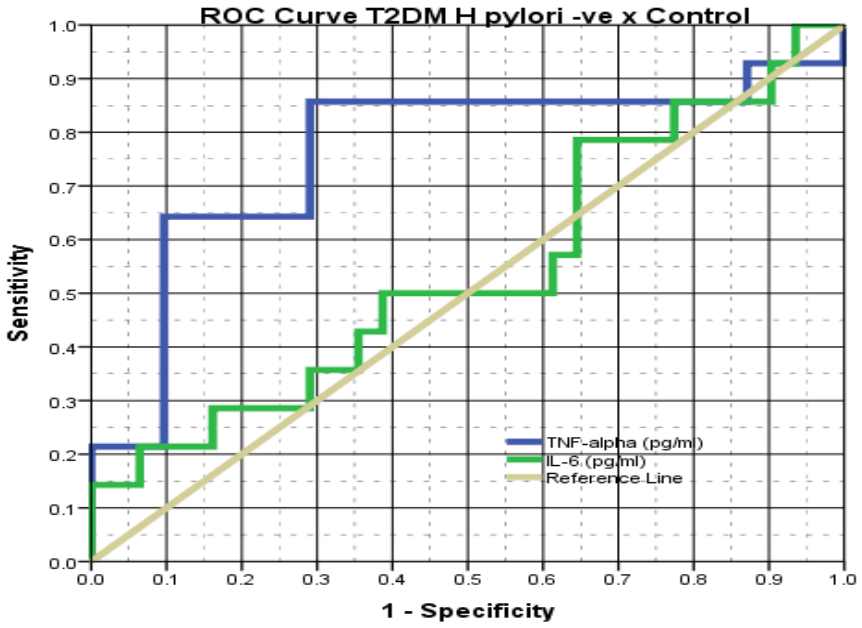


Figure.4 Receiver operator curve (ROC) analysis for the investigated parameters in T2DM -ve *H.pylori* patients and controls



The present study was performed on a pre diagnosed T2DM patients with no signs and symptoms of *gastrointestinal abnormalities* to investigate the effect of infection with *H. pylori Cag A* positive bacteria on cytokine levels,glucose tolerance and lipid profile which thought to be involved in T2DM development.The results obtained and compared between control individuals and T2DM patients and among *the* (+ve and -ve *H. pylori Cag* A) T2DM patients.In addition,the correlation between *Cag* A seropsitivity and those markers were determined.

The earlier studies approved a close association between inflammation and presence of *H. pylori* (Cong H, 2014; Fariba Shojaee-Moradie, 2013; Holck, S, 2003). The inflammation induced by *H. pylori* in the gastrointestinal region leading to increase glucose and lipids absorption, that are also not normal in case of diabetes mellitus(Cong H, 2014).However, no constituting information's approving that *H. pylori* have a role in the development of diabetes, the possible causal effect of *H. pylori* is an interesting theory needing further investigation (Lahner E, 2002).

In our study,We measure the level of TNF-α and IL-6 as a markers of inflammation due to their implication in insulin resistance and development of diabetes(Wellen KE, 2005).

The current study recorded a significant increase in TNF- α level was in *(H. pylori* +ve patients) T2DM patients group as compared to healthy group, while there was a non significant elevation in TNF- α level in *H. pylori* -ve patients T2DM as compared to its mean level in *H. pylori* +ve T2DM patients groups and in healthy group. But, the difference was not significant between +ve and –ve *H. pylori* T2DM patients groups.

However, the TNF-α ROC curve show different shape when compared between +ve and -ve *H. pylori* T2DM patients groups.

Those findings were agreed with other study preformed that *H. pylori* infection is accompanied with a significant increase in TNF-α secretion and this increase lead to stimulation of cell apoptosis (Takagi A, 2000).This expressed with other study which was conducted to see the action of *H. pylori* on cell apoptosis and proliferation in the lines of epithelial cells of the gastric mucosa (Aliment, 2002).

A previous study shown that the increasing in TNF-α secretion by *H. pylori* was independent on the expression of Cag A status or Vac toxin (Takagi A, 2000) .

The suggested pathway for TNF-α elevation is the production of mediator proteins by *H. pylori* that stimulate the activity of NF-κB (Takagi A, 2000). This proinflamatory mediator plays a key role in regulating the immune response and cytokine expression during the infection(Lawrence T, 2009). NF-κB shown to be increase and result in stimulation of TNF-α secretion in *H. pylori*-associated gastritis according to many studies (Takagi A, 2000 ; Ikhlas K, 2012).

Other suggested findings demonstrated that *H. pylori* stimulate interferon - gamma secretion by T-cells stimulation(Ren, Z., 2000). Ideas about an interaction between TNF-alpha and IFN -gamma might be necessary for Helicobacter pathogenesisty same as approved for gastritis induced by *H. felis* infection (Blaser MJ, 2004).

Thalmaier and others using cknockout mice for analysis of the immune pathogenesis and

cytokine expression on *H. pylori* infection, They were reported that in contrast to TNF-α signals mediated by the TNF-R1 pathway, IFN-γ plays a major role in the induction of gastric inflammation caused by *H. pylori* infection. Also, These results concluded that TNF-α mediated by the TNF-R1 pathway is critical in maturation of the systemic humoral immune response, with formation of primary B-cell activation, B-cell follicles, and production of IgG antibodies (at least IgG1, IgG2b, and IgG3) (Thalmaier, 2002).

TNF-α was found to be significantly elevated in diabetic subjects versus the control group, also there was a significant difference in TNF-α level in T2DM *H. pylori* +ve versus T2DM *H. pylori* -ve group. IL-6 was found to be high in T2DM group when compared to healthy subjects and there was a significant difference in IL-6 level between control and T2DM *H. pylori* +ve patients groups.

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