

**DETECTION OF CYTOTOXIC T LYMPHOCYTE ANTIGEN -4 (CTLA4) IN TYPE 1 DIABETES PATIENTS IN IRAQ BY ELISA.****Haifa M. Fahad\***

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Iraq.**ABSTRACT**

**Background:** CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4), also known as CD152, is a protein that acts as an important regulator of the immune system. This study aimed to detect of CTLA4 in type1 diabetes patients (T1D), and compared with healthy control subjects, and determination of GAD autoantibody. **Materials and Methods :-** 66 Iraqi T1D patients who were referred to ALKindy Specialized Center For Diabetes and Endocrinology and the Central Child Hospital and 20 blood samples from healthy control were enrolled in this study during the period between June to the end of December 2012. Their

ages between(1-18 year), are divided into three groups, newly diagnosed, chronic patients and healthy control groups. All cases were subjected to a thorough history taking, full clinical examinations and investigations which include; glutamic acid decarboxylase (GAD) autoantibody levels and CTLA4 detection by using ELISA. **Results:** GAD autoantibody level was significantly higher in patient groups (newly diagnosed and chronic patients) compared with healthy controls. The serum level of (CTLA-4) in serum samples was, appeared to have a low concentration in chronic patients, and newly diagnosed compared with healthy control, while the correlation between CTLA4 and GAD autoantibody was non significant. **Conclusion:** High concentration of GAD autoantibody was found in the most of T1D patients and is a very robust tools for predication of developing of T1D, and low concentration of CTLA-4 antigen was found in T1D patients.

**KEYWORDS:** CTLA-4, T1D, GAD, ELISA.**INTRODUCTION**

Type1diabetes (T1D) is an autoimmune disease in which an inappropriate self-directed immune response affects and destroys insulin-producing beta-cells in pancreatic islets leading

to dysregulated blood glucose levels. Also T1D is a T cell – mediated, chronic disease characterized by a deficient or absence of insulin, when the body's own immune system attacks the  $\beta$  cells in the islets of Langerhans of the pancreas (Andrea *et al.*, 2013; Andras *et al.*, 2013). CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4), also known as CD152, is a protein that acts as an important regulator of the immune system. CTLA4 is a member of the immunoglobulin superfamily, which is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells by binding with B7(CD80) on antigen presenting cell and initiate a negative feedback signal that can stop the activation signal, proliferation and the survival of T cells (Pruul *et al.*, 2013). The markers of the immune destruction of the beta cells include islet cell autoantibodies, autoantibodies to glutamic acid decarboxylase. These autoantibodies are present in 85-90% of individuals with type 1 diabetes (ADA, 2011), and the glutamic acid decarboxylase (GAD) is a major autoantigen involved in the pathogenesis of type 1 diabetes mellitus, and GAD is one of the major antigens targeted by self reactive T-cells in T1D (Stina, 2012; Yuichiro *et al.*, 2012).

The aim of this study is to detection of CTLA4 in type 1 diabetes patients (T1D), and compared with healthy control subjects, and determination of GAD autoantibody.

## MATERIAL AND METHODS

### Subjects and Methods

#### Patient Group

This group included 66 subjects for CTLA4 and for GAD test those were suffering from T1D after fasting blood glucose and HbA1c tests were done in a period between June and the end of December 2012. They were selected from the AL-Kindy Specialized Center for Diabetes and Endocrinology and the Central Child Hospital. Their ages ranged from 1-18 years, with mean age of ( 7.7 years  $\pm$  4.2 ) for short duration  $\leq$  3 months (newly diagnosed) group and mean age of (11.75  $\pm$  4.19 years ) for long duration  $>$ 3 months (chronic) group.

#### Healthy control group

Twenty healthy individuals with no family history of diabetes were considered as a control group. They were free from any systemic disease. Their ages ranged from 5-16 years with a mean age of (11.5  $\pm$  6.3 years).

### Laboratory analysis

Venous blood (3 ml) was withdrawn from each subject of the three groups. Blood was left to clot for 15 minutes, centrifuged and serum was separated some was used immediately for a glucose oxidase method of fasting blood glucose (FBG) analysis (supplied by GLU-PAP/United Kingdom), and measurement absorbance at 500 nm by using spectrophotometer. The remaining serum was kept frozen at  $-20^{\circ}\text{C}$  for the subsequent assay using by Enzyme Immunoassays for the quantitative determination of CTLA4 Ag and GAD Ab in human serum using a kit supplied by (Cusabio, China) for CTLA4 and (RSR, United Kingdom) for GAD ELISA Kit.

### Statistical analysis

Statistical Analysis System – SAS (2010) program was used to detect the effect of difference factors in study parameters. The linear relationship between variables was assessed by Pearson's correlation coefficient (r). For all tests, P values less than 0.05 were considered statistically significant.

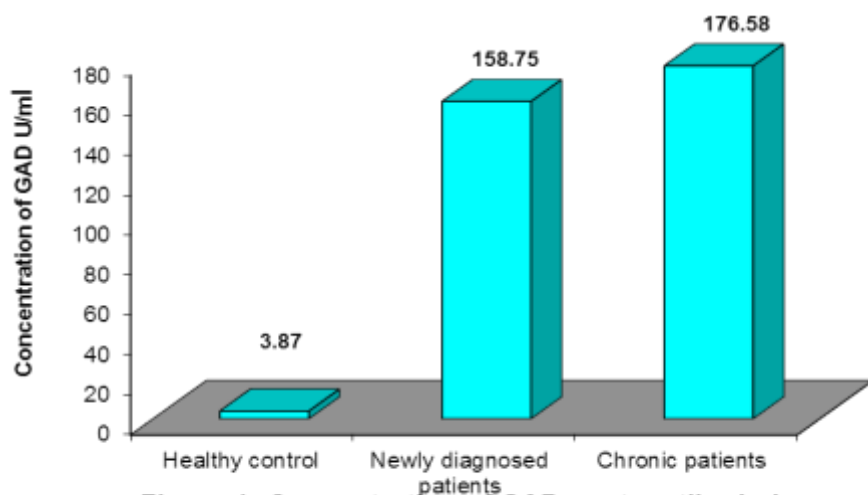
## RESULTS

Table (1) figure (1) showed the mean  $\pm$  SE of glutamic acid decarboxylase autoantibody concentration for the study groups. There was a significant differences between patients groups (newly diagnosed and chronic patients) ( $158.75 \pm 34.4$ ;  $176.58 \pm 48.16$ ) respectively, when compared with healthy control ( $3.87 \pm 0.18$ ). In figure (1) demonstrated the highest concentration of GAD autoantibody in chronic patients group when compared with healthy control group according to cut off point negative  $< 5 \geq$  positive.

**Table 1:- The concentration of GAD-autoantibody in serum of study groups of T1D and healthy subjects.**

Study groups	N	%	CONCENTRATION U/ml Mean $\pm$ SE
Healthy control	20	23.2%	$3.87 \pm 0.18^b$
Newly diagnosed patients	33	38.3%	$158.75 \pm 34.4^a$
Chronic patients	33	38.3%	$176.58 \pm 48.16^a$

(a,b) Small letters denoted that is high Significant differences between groups of study at ( $p \leq 0.05$ ). Assay Cut off, negative  $< 5 \geq$  positive.



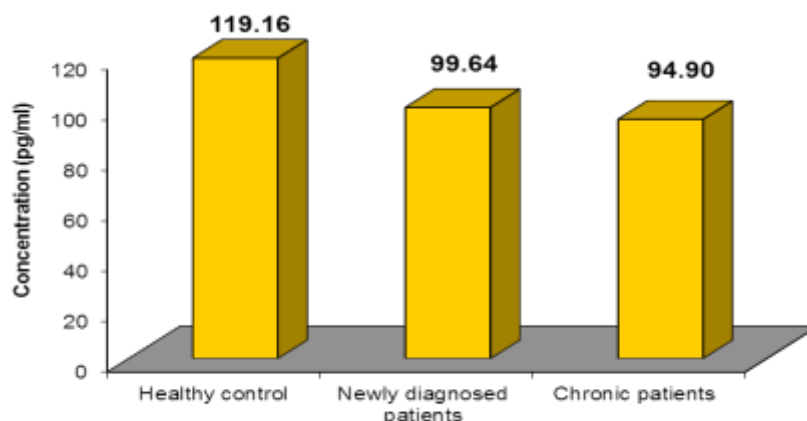
**Figure 1: Concentration of GAD –autoantibody in serum of study groups**

Table (2) and Figure(2) showed the concentration of Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) antigen in serum of study groups, was significantly decreased in patients groups ( $99.6 \pm 5.1$  pg/ml ;  $94.9 \pm 4.45$  pg/ml) when compared with the healthy control group ( $119.16 \pm 9.90$  pg/ml) respectively.

**Table 2:- The concentration of CTLA-4 in serum of study groups of T1D using ELISA.**

GROUPS	N	CONCENTRATION pg/ml Mean $\pm$ SE
Healthy control	20	$119.16 \pm 9.90^a$
Newly diagnosed patients	33	$99.64 \pm 5.17^b$
Chronic patients	33	$94.90 \pm 4.45^b$

(a,b) Small letters denoted that is Significant differences between groups of study at ( $p \leq 0.05$ ).



**Figure 2: The concentration of CTLA-4 in serum of study groups using ELISA**

Table 3 explained the correlation between GAD and CTLA4 . The result showed the negative correlation was non significant.

**Table(3):- The Correlation between CTLA-4 with GAD autoantibody in type1 diabetes patients.**

<b>Marker</b>	<b>CTLA-4</b>	<b>Sig.</b>
<b>Parameter</b>		
GAD	-0.033	NS
NS=Non significant		

## DISCUSSION

This is a first study of the detection of Cytotoxic T-Lymphocyte antigen-4 (CTLA-4) in serum of type1 diabetes patients in Iraq .CTLA-4 is known as a major down- regulator of immune responses by preventing T- cells activation and down – regulating T-cell expansion (Paula *et al.*,2009), and CTLA-4 is a member of immunoglobulin super family that is expressed on the surface of activated T-cells (Snchneider *et al.*,2006 & Lemos *et al.*, 2009).Also CTLA-4 may have important immunoregulatory functions, its effect might depend on the activation state of the cells involved, CTLA-4 could block CD80/CD86- CD28 interactions, interfering with T-cell co stimulation on the opposite, inhibition of CD80/CD86- CTLA-4 interactions on activated T-cells may prevent down- regulation of T-cell responses . Such hypothesis is supported by previous studies ( Peng and Hagopian,2006 ;Saverino *et al.*,2007; D Angeli *et al.*,2010 and Stina,2012) . The results showed a significant decrease between patients and healthy control. The low concentration was found in diabetic groups compared with the concentration of healthy control. The results of this study are in agreement with Anna's study,(2011) who observed low level of circulating soluble CTLA-4 in T1D patients ,and disagreed with Simone *et al.*,(2009)and Momin,(2009) who found an increase of CTLA-4 in the sera of autoimmune T1D when compared with healthy controls. The lower level of CTLA-4 may be due to the interaction of CD80 and CTLA-4 in serum. This result agreed with study of Pruul *et al.*,(2013) who found that newly diagnosed T1D among children and adolescents is associated with activation of CD28 and CTLA-4 in serum. In addition of previous studies of Dul *et al.*,(2009); Wafai *et al.*,(2011) & Najwa *et al.*,(2012) were observed CTLA-4 plays a role in limiting T cells proliferative by the balance between CD28 and CTLA-4 interactions with B7 could lead to autoimmune disease by preventing apoptosis or down- regulation of activated self reactive T-Lymphocyte .The reason of lower levels of CTLA-4 may be due to the 49+A/G polymorphisms that have been connected to the

potential reduction of s CTLA-4 in T1D patients carrying the G allele, this confirmed with the results of the previous studies of Kay *et al.*,(2011); Tom *et al.*,(2011) and Schiavo & Saverino,(2013), which found in the human the CTLA-4 promotor region and in exon1 the A49G polymorphism is the only polymorphism that changes the primary amino acid sequence of CTLA-4 . The American Diabetes Association defined patients with type1 diabetes as those who have an immunologic disorder such as GAD antibody positive Hui *et al.*,(2013). Which may be used as an earlier marker than FBG and are very robust tools for predication of developing of T1D. Hassan ,(2008)who studied of the Iraqi patients who found the seropositivity for GAD antibody was high, which indicated the progressive nature of the autoantibody stimulation which seems to be in consistency with the level of  $\beta$ - cell destruction . In comparison a high GAD seropositivity was reported for T1D patients group in this study which verifies the suspected role played by this antibody in the pathogenesis of the disease, and was similar to Iraqi study of Madha *et al.*,(2012) who reported that GAD antibody in patients was high. Whereas, it disagreed with Sergio and Marilia,(2009) who explained that the presence of these autoantibody for at least 3 to 6 months indicate the presence of autoimmunity against pancreatic islets, which was confirmed in the present study.

## CONCLUSIONS

This study concluded that patients with T1D have high concentration of GAD autoantibody was found in the most of T1D patients and is a very robust tools for predication of developing of T1D, and low concentration of CTLA-4 antigen was found in T1D patients.

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