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Evaluation of ADA, IL-6 and TNF-alpha level in type 2 diabetes mellitus: with -and without hypoglycemic drugs.

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Abstract
Diabetes mellitus(DM) is a major worldwide health problem leading to markedly increase mortality and serious morbidity. Immunological disturbances involving the cell mediated immune system and improper T-lymphocyte function also contribute to the path physiology of type 2 DM.It has been reported that ADA,IL-6,and TNF-α levels were a good marker for immunological disturbance in type 2 DM patients.This study aims to assess and compare the level of serum ADA, IL-6 ,and TNF-α in patient of type 2 DM with and without oral hypoglycamic drugs.The study population consist of 150 subjects divided in to 3 groups:group I (50normal health controls),group II (45 type 2 DM patients with no on hypoglycemic drugs),and group III (55 type 2 DM patients on hypoglycemic drugs).There were a significant($p<0.001$) tremendous increase in ADA,IL-6,and TNF-α levels (47.32 U/L ,29.04 pg/ml ,and 98.23 pg/ml, respectively) in group II than group I and group III. also, ADA,IL-6,and TNF-α levels were significantly($p<0.001$) higher in group III than group I.As conclusion ,the increase in ADA,IL-6,and TNF-α levels is a good glycemic markers associated with type 2 DM .The intake of hypoglycaemic drugs decrease the levels of these markers.
Key words: ADA activity , IL-6 , TNF-α , type 2 diabetes mellitus

1.Introduction

Diabetes mellitus is the major health problem affecting both developed and developing countries, its a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both(Bobb etal.2008).The chronic hyperglycemia of diabetes is associated with long-term dysfunction, damage, and failure of various organs, especially the eyes,kidneys,nerves, heart, and blood vessels(Li *et al*.,2011).

Type 2 diabetes mellitus, the most prevalent form of the disease, is often asymptomatic in its early stages and can remain undiagnosed for many years. Individuals with undiagnosed type 2 diabetes are also at significantly higher risk for stroke, coronary heart disease, and peripheral vascular disease than the non diabetic population(ADA,2008).Insulin resistance and impaired insulin secretion are the main physiological abnormalities associated with T2DM (Khemka *et al.,*2013). Immunological disturbances involving the cell mediated immune system and improper T-lymphocyte function also contribute to the pathophysiology of T2DM (Prakash et al.2006)

Adenosine deaminase (ADA), an enzyme presents in red cells and the vessel wall catalyses the irreversible hydrolytic deamination of adenosine to inosine and 2'- deoxyadenosine to 2'-deoxyinosine.Both inosine and 2'-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid (UA. The enzyme exists in two isoenzyme forms:(ADA1 and ADA2) which are coded by separate genes (Gakis,1996). ADA is considered as a good marker of cell mediated immunity. Adenosine is a suppressant of inflammation;effectively inhibit T-cell activation and proliferation.Increased level of ADA decreases adenosine concentration.Increased ADA indicates increased inflammatory state of a cell Its play a crucial role in lymphocyte proliferation and differentiation and its shows its highest activity in T-lymphocytes (Sullivan *et al*.,1977).High lymphocyte ADA activities were found to be elevated in diseases in which there is a cell mediated immune response (Prakash *et al*.2006).

Adenosine deaminase (ADA) is an enzyme that has been suggested to be important for modulating the bioactivity of insulin (1) Chronic hyperglycemia leads to increased oxidative stress by forming enediol radicals and superoxide ions by NADPH oxidase system and increases ADA levels, both leading to insulin resistance(Singh *et al* ,2013).

Individuals who progress to type 2 diabetes display features of low-grade inflammation years in advance of disease onset.This low-grade inflammation has been proposed to be involved in the pathogenetic processes causing type 2 diabetes.Mediators of inflammation such as tumor necrosis factor, interleukin ,IL-6, IL-18,and certain chemokines have been proposed to be involved in the events causing both forms of diabetes. IL-6 has in addition to its immunoregulatory actions been proposed to affect glucose homeostasis and metabolism directly and indirectly by action on skeletal muscle cells,adipocytes, hepatocytes,pancreatic-cells, and neuroendocrine cells (Kristiansen and Mandrup-poulsen,2005).

Concentrations of acute-phase response markers and mediators of inflammation such as cytokines like TNF-α and IL-6 are also raised in people with T2DM. This finding has led to the suggestion that raised concentrations of pro-inflammatory cytokines and the resultant acute-phase response may underlie much of the metabolic

clustering due to diabetes mellitus(Hotanisligil *et al.,*1994). cytokines operate as a network in stimulating the production of acute-phase proteins. Obesity, as well as hyperglycemia, contributes to the rise of these inflammatory markers(Goyal, *et al.*2015).

The present study was undertaken to assess and compare level of serum ADA activity and serum IL-6 and TNF-α in patients with type 2 diabetes mellitus with and without treatment in order to use it as a marker of immunological disturbances in T2DM patients.

2. Materials and Methods

The subject included in the present study were 100 patients of type2 DM (55 of them on oral hypoglycaemic drugs and 45 of them without on any hypoglycemic drugs) with age group of 25-60 years of either sex. A group of 50 normal healthy individuals ,who were age and sex match with patients group.the study group and control were selected from patient and healthy individuals visiting the clinical lab.of AL-Sadder Medical City in Najaf/ Iraq during the period 2015. Five ml of venous blood were collected from patients and control by vein puncture and allowed to clot at room temperature for 30 min.then centerfuged for 20 minutes at approximately 1,000 x g.and then The serum separated for ADA ,IL-6,TNF-α level detection. These 150 subjects were divided into three groups:

Group I: Comprised of 50 normal healthy individuals as control group.

Group II: Comprised of 45patients of types 2 DM without hypoglycaemic drugs.

Group III: Comprised of 55 patients of types 2 DM on hypoglycaemic drugs.

Inclusion Criteria: All clinically diagnosed cases of type 2 DM were included in the study was based on 2010 American diabetic associated criteria (27) many patients with history of type 2 DM for more than 1 years were included the study.

2.1 Exlusion Criteria

Patients with type 2 DM with any other chronic disease such as renal failure,Rheumatoid arthritis,tuber culossis, and patients with type 2 DM on insulin treatment, to minimize possible confounding of results.

The total level of serum ADA was measured by end point colorimetic method by commercially supplied Kit (Tulip Diagnostic).the assay was based on the colorimetric method described by Gusti and Galanti (Guisti,1974).

The concentration of TNF-α and IL-6 in serum samples of type 2 DM and healthy controls were done by use of a commercial ELISA Kit (Orenium Laboratory,Finland).The interassay and intra coefficients of variation for IL-6 were 9.4 % and 8.8 % and for TNF-α were 5.2% and 7.4%.

2.2 Statically analysis

The results obtained from this study were expressed as mean± SD by using Excel 2007.Ttest was done to compare ADA,TNF-α ,and IL-6 between three groups by using SPSS Software, Version 16.

3. Results and Discussion

In the present study,the mean serum ADA level of group II (patients of type 2 DM without any hypoglycaemic drugs) were significantly higher ($P< 0.001$) than group I (control group) and group III (patients of type 2 DM on oral hypoglycaemic drugs).Also, the level of ADA were significantly higher in group III (30.05) U/L than group I(18.20) U/L (Table 1).

Diabetes mellitus,a common endocrine metabolic disorder, is a leading cause of death worldwide. It is characterized by hyperglycemia resulting from a variable interaction of hereditary and environmentalfactors and is due to the combination of insulin resistance (impairment in insulin-mediated glucose disposal) and defective secretion of insulin by pancreatic β cells (WHO,2014).

In the present study, patients of type 2 DM (group I and group ΙΙΙ)appeared high level of ADA than normal healthy control. Similar results were reported by Hoshino *et al*.,(1994), Kurtol *et al*.,(2004), Kaur *et al*., (2012), and Singh *et al*., (2013). All these study reported elevated ADA level in serum of type 2 DM patient whereas Angielski *et al*., (1989) demonstrated that ADA activety were not change in isolated glomeruli of Streptozocin diabetic rats. Ramani *et al.*,(2012) depleted that ADA activity in type 2 DM patients was significant highes than in the control group(mean ± SD of 32.06 ± 17.09 v. 19.28 ± 5.59, $p< 0.001$). Shantaram *et al*.(2014).found that ADA level were decreased in the patients of type 2 DM as compared to controls. And they found that the decrease in ADA level may be due to the depressed cell mediated immunity in the patients due to intake of medicines to control their blood sugar.

ADA plays a crucial role in lymphocyte proliferation and differentiation , and shows its highest activity in T-lymphocytes . In the present study, a significant elevation in the ADA levels was observed in diabetic subjects compared to the controls. High plasma ADA activity might be due to abnormal Tlymphocyte responses or proliferation and may point to a mechanism that involves its release into circulation (Ankush *et al.,*2009).Therefore, as presented in Table (1), increased ADA activity in diabetic individuals could be due to altered insulin related T-lymphocyte function (Kumar *et al*.,2008).

Insulin resistance and impaired insulin secretion are the main physiological abnormalities associated with T2DM .Adenosine is responsible for increasing glucose uptake into cells.Thus, higher ADA activity in insulin sensitive tissue will decrease adenosine levels which in turn decrease glucose uptake into cells.ADA also plays a crucial role in lymphocyte proliferation and differentiation and is highly active in T-lymphocytes (Zavialov *et al.,*2010).Thus, a suppression of ADA activity may help improve insulin sensitivity and inflammation,cell proliferation, and T-lymphocyte activity,all of which are putatively associated with the pathophysiology of T2DM.

In other words,Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and the myocardium, while it inhibits the effect of insulin on total hepatic glucose output, which suggests that adenosine, causes local insulin resistance in the liver. Adenosine modulates the action of insulin on various tissues differently and its concentration in tissues is affected by ADA level (Warrier *et al.,* 1995).

In this study, anegligible level of TNF-α (5.22 pg/ml) and IL-6 pg/ml) was recorded in group I (normal healthy control).Sample of group III (type 2 DM patient without any hypoglycaemic drugs) showed an significant ($p< 0.001$) augmentation in the level TNF-α (24.7 pg/ml) and IL-6 12-4 pg/ml),respectively.A tremendous augmentation was observe in the level of TNF-α (98.23 pg/ml),$p< 0.001$) and IL-6 (29.04 pg/ml) in group II (type 2 DM patients on oral hypoglycemic drug).

The result of this study compatible with Goyal *et al*,2015) when they reported an increase of TNF-α and IL-6 level in DM patients and increase was more in obese than nonobes diabetics), and also, they found that insulin induced down regulation of TNF-α and IL-6 in nonobes and obese diabetical patients.

Ahmed (2005) and Goldsby *et al*.,(2008) reported that Hyperglycaemia causes formation of Advanced glycation End Products (AGEs).The AGEs stimulate receptors for advanced glycation end products (RAGE).In addition they increase the generation of reactive oxygen species in macrophages thereby causing heightened oxidative stress. AGEs bind to AGE receptors on several cell types (endothelial cells, mesangial cells and macrophages) lead to release of cytokines; TNF-α, IL-1,IL-6 and growth factor from macrophages and mesangial cells resulting in activation of T lymphocytes.Furthermore, in the presence of superoxide dismutase, superoxide anion leads to formation of H2O2 which is responsible for activating the signalling molecules leading to inflammation (Singh *et al*., 2009).In addition,A close correlation has been found between the severity of inflammation and a local increase in both expression and activity of ADA (Kaur *et al*.,2012).

TNFa and IL-6 are the major cytokines produced by adipose tissue and their circulating levels are increased in diabetics(Rajarajeswari *et al*.,2011).Elevated levels of IL-6, which is the main stimulator of the production of most acute-phase proteins,increase the risk of diabetes(Pradhan *et al*.,2001).Goyal *et al.* (2015) found that insulin-induced down regulation of TNF-α and IL-6 in nonobese and obese diabetic patients. Therefore, we concluded that elevated adenosine deaminase and pro-inflamatory cytokines(TNF-α and IL-6) my be an important indicator in the immunopathogenic of type 2 diabetes mellitus .

Table 1.Comparison of serum Adenosine Deaminase (ADA) levels in three groups

Group	Number	Mean ± SD (U/L)	Comparison	P value
I	50	18.20 ± 6.21	I *vs.* II	*P< 0.001*
II	45	47.32 ± 8.79	I *vs.* III	*P< 0.001*
III	55	30.05 ± 12.14	II *vs.* III	*P< 0.001*

Table 2.Comparesion of TNF-α and IL-6 level in three groups

Group	TNF-α (pg/ml)	IL-6 (pg/ml)
I	5.32 ± 1.50	6.64 ± 1.23
II	98.23 ± 3.2	29.04 ± 1.0
III	24.7 ± 1.45	12.4 ± 2.8
P value	*P< 0.001*	*P< 0.001*

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