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Study of Some Immunological Physiological and Biochemical Parameters in Patients with Type 1 Diabetes Mellitus in Kirkuk City

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Abstract

This study was designed in order to estimate some immunological (IL-6), physiological (Resistin) and biochemical (Malondialdehyde and Glutathione) parameters of some patients with type 1 diabetes mellitus in Kirkuk city. The study involved (60) blood samples were divided into 2 groups: group one: normal healthy individuals as control group, group two: patients with type 1 diabetes mellitus. The results of this study found significant increase in pro-inflammatory cytokine IL-6 concentrations at levels ($P \leq 0.05$) of second group in comparison with control group ; and there are significant increases in resistin concentrations at levels ($P \leq 0.05$) of second group in comparison with control group; and there are significant decreases in glutathione (GSH) concentrations at levels ($P \leq 0.05$) of second group in comparison with control group, and there are significant increases in malondialdehyde (MDA) concentrations at levels ($P \leq 0.05$) of second group in comparison with control group.

دراسة بعض المتغيرات المناعية و الفسلجية والكيموحيوية لدى مرضى داء السكري (النوع الاول) في مدينة كركوك

الخلاصة

صممت هذه الدراسة لتقييم بعض المتغيرات المناعية (انترليوكين- ٦)، الفسلجية (الرزسستين) والكيموحيوية (المالوندايالديهايد و الغلوتاثايون) لبعض المرضى المصابين بداء السكر النوع الاول في مدينة كركوك . شملت الدراسة ٦٠ عينة دم والتي قسمت على مجموعتين : المجموعة الاولى : افراد اصحاء كمجموعة سيطرة , المجموعة الثانية : مرضى بداء السكر النوع الاول . وجدت نتائج هذه الدراسة ارتفاع معنوي في تراكيز الحركي الخلوي الممهد للالتهاب انترليوكين-٦ عند مستويات ($P\leq 0.05$) لدى المجموعة الثانية عند المقارنة مع مجموعة السيطرة ، وهناك ارتفاع معنوي في تراكيز الرزسستين عند مستويات ($P\leq 0.05$) لدى المجموعة الثانية عند المقارنة مع مجموعة السيطرة ، وهناك انخفاض معنوي في تركيز الغلوتاثايون عند مستويات ($P\leq$ 0.05) لدى المجموعة الثانية عند المقارنة مع مجموعة السيطرة ، بينما هناك ارتفاع معنوي في تركيز المالوندايالديهايد عند مستويات ($P\leq 0.05$) لدى المجموعة الثانية عند المقارنة مع مجموعة السيطرة.

Introduction

Type 1 Diabetes (T1D) is a potentially life-threatening multifactorial autoimmune disorder characterized by T-cell-mediated destruction of pancreatic β cells with leukocytes infiltration , resulting in a deficiency of insulin synthesis and secretion ; potential triggers of islet autoimmunity in addition to genetic susceptibility include infections , early life diet , vitamin D levels , gut microbe composition , vaccinations and pollutants and toxins [1 , 2] .

IL-6 is 26 kDa is four-helical glycopeptide pleiotropic cytokine mediates various biological functions, including regulation of the immune system, regenerative processes, metabolism, bone homeostasis, cardiovascular protection and neural function. IL-6 plays an important role in the development and activation of both innate and adaptive immune system.[3]. IL-6 is an important pro-inflammatory cytokine in conditions of inflammation, infection and autoimmunity by which many inflammatory responses in tissues are initiated by IL- 6, which promotes the infiltration and activation of mononuclear white blood cells while suppressing neutrophil infiltration ; furthermore, IL-6 signaling is associated with the upregulation of anti-apoptotic factors that promote T cell survival. IL-6 also regulate immunoglobulins synthesis by which IL-6 induces the differentiation of activated B cells into plasma cells , additionally IL- 6 promotes the differentiation of T follicular helper cells [4]. IL-6 is a key pathogenic cytokine in multiple autoimmune diseases including rheumatoid arthritis and multiple sclerosis, suggesting that dysregulation of the IL-6 pathway may be a common feature of autoimmunity including type 1 diabetes by which signal transducer and activator of transcription 3 (STAT3) and STAT1 responses to IL-6 are significantly enhanced in CD4 and CD8 T cells in type 1 diabetes patients . and dysregulation of IL-6 signaling may be a marker of early disease and contribute to type 1 diabetes through multiple mechanisms including altered T cell trafficking [5].

Resistin is an adipocyte-specific hormone of 12.5 kDa (108 amino acids). Resistin initiate inflammatory processes. The main physiological role of resistin may be to modulate the inflammatory, immune, and autoimmune responses During pathological inflammation, the release of resistin by infiltrated monocytes/macrophages is incited by pro-inflammatory cytokines such as C-reactive protein (CRP), IL-1, IL-6, IL-12, and TNF-α (through the activation of NF-kB). Resistin affects a wide range of cells and tissues via autocrine, paracrine, and endocrine mechanisms, enhancing the Th1 immune response as well [6]. Resistin, is an important link between obesity, insulin resistance and diabetes. Resistin plays important regulatory roles apart from its role in insulin resistance and diabetes in a variety of biological processes: atherosclerosis and cardiovascular disease, non-alcoholic fatty liver disease, autoimmune disease, malignancy, asthma, inflammatory bowel disease and chronic kidney disease [7 , 8]
Malondialdehyde (MDA) is a highly toxic by-product formed by lipid oxidation induced by free radicals. MDA is the main metabolite of arachidonic acid reacting with proteins and phospholipids [9].Malondialdehyde is increase in the serum of type 1 diabetes patients because there is increase in oxidative stress in T1D which causes lipid peroxidation ; thus MDA is a marker of lipid peroxidation helps to measure the overall oxidative stress in this disease [10].

Glutathione (GSH), is a water soluble endogenous tripeptide, from three amino acids glycine, cysteine, and glutamic acid [11]. GSH is potent antioxidant protecting the cell from the oxidative stress, and act as cofactor for Glutathione peroxidase (GPx) which is a defensive mechanism against peroxides,

preventing the accumulation of reactive oxygen species (ROS) and so preventing cellular injury [12].

Material and Method

Patients and Blood Collection

This study was done in the period from August 2021 to December 2021 involving 60 blood samples with 30 blood samples from patients diagnosed with type 1 diabetes mellitus with age range (6-15) year. The blood samples are collected from external laboratories in Kirkuk city and divided into two groups: First group include 30 blood samples of type 1 diabetes mellitus patients, second group include 30 blood samples of healthy normal individuals as control group. Collection of blood samples was prepared from (5 ml) venous blood obtained by using disposable syringe and clean dry plain tubes without any anticoagulants and left it at room temperature to coagulate. After that centrifuged for ten minutes at 4000 rpm to get serum without any hemolysis.

Determination of parameters

Serum malondialdehyde was measured according to modified method used by (Guidet and Shah, 1989) [13].Glutathione was estimated by the modified method used by (Tietz, 1999) [14]. IL-6 and resistin were determined by using its kit from (MyBioSourse) company (USA) of ELISA technique.

Statistical Analysis

The data were analyzed by (SAS, 2001) software according to one way ANOVA followed by Duncan range test used at a statistical level of (p 0.05).

Results and Discussion:

This study showed significant elevation in fasting blood glucose concentration (FBG) ($p \leq 0.05$) in type 1 diabetes group during the experiment period Figure (1) (161.00 ± 17.24 mg/dl) as compared with control group .

These results coincide with the studies of Fatima *et al.* (2016) [15]; they found significant increase of blood glucose in patients with type 1 diabetes in comparison with control group.

This study showed significant raising in blood serum pro-inflammatory cytokine IL-6 concentrations ($p \leq 0.05$) in type 1 diabetes group during the experiment period Figure (2) (29.14± 6.22 pg/ml) as compared with control group.

These results agree with the studies of Al-Taee and Al-Noaman (2018) [16] and Hameed *et al.* (2012) [17] , they found significant increase of blood serum IL-6 in patients with type 1 diabetes in comparison with control group.

IL-6 is a marker of inflammatory process as it alter insulin secretion through direct or through stimulation of free fatty acid production and altered glucose homeostasis thus IL-6 is important factor in the immune system and has important role in glucose metabolism ; furthermore hyperglycemia in type 1 diabetes stimulates the, monocytes to secrete increased amounts of IL-6 via upregulation of Protein kinase C , mitogen-activated protein kinase and Nuclear factor (NF-κB) activity, leading to increased IL-6 transcription and release [16 , 17].

This study showed significant increase in blood serum adipokine resistin concentrations ($p \leq 0.05$) in type 1 diabetes group during the experiment period Figure (3) (6.12 ± 1.35 ng/ml) as compared with control group.

These results in agreement with the studies of İclal *et al.* (2013) [18] and Yazici *et al.* (2012) [19] , they found significant increase of blood serum resistin in type 1 diabetic patients in comparison with control group.

Resistin is a putative adipocyte-derived signaling polypeptide , majority of the resistin expression and secretion from adipose tissue is found in stromal vascular fraction including undifferentiated preadipocytes, vascular endothelial and smooth muscle cells, and in some cases immune system cells such as monocytes and macrophages , resistin

link obesity with insulin resistance and diabetes resistin is greatly implicated in proinflammatory processes which are causally involved in the development of insulin resistance (IR) in both rodents and humans. Resistin may interfere with insulin signaling by stimulating the expression of phosphatase and tensin homolog deleted on chromosome ten (PTEN), which dephosphorylates 3-phosphorylated phosphoinositide (PIP3). This leads to the decrease in PDK and Akt phosphorylation ; another possible mechanism is that resistin inhibited adipocyte differentiation by 80% , thus resistin may promote insulin resistance by increasing the storage of triglycerides in muscle and liver instead of adipose tissue [20 , 21 , 22]. This study revealed significant elevation in blood serum Malondialdehyde (MDA) concentrations ($p \leq 0.05$) in type 1 diabetes group during the experiment period Figure (4) (3.78±0.64 µmol/L) as compared with control group. These results coincide with the studies of Reis *et al.* (2012) [23] and Hoeldtke *et al.* (2011) [24] , they found significant increase of blood serum malondialdehyde (MDA) in type 1 diabetic patients in comparison with control group. MDA produced due to lipid peroxidation occurs by the oxidation of lipids via free radicals of Oxygen ; MDA may also be produced in high levels as a byproduct of thromboxane synthesis , degradation of endoperoxides and cycloxgenase reactions [25] ; In type 1 diabetes there is elevation in MDA levels because there is chronic hyperglycaemia causes chemical alterations in proteins, lipids and DNA and also facilitate generation of reactive oxygen species (ROS) mediated enhanced oxidative stress including lipid peroxidation and accompanied by impaired antioxidant defense [15].

The study showed significant decrease in blood serum glutathione (GSH) concentrations ($p \leq 0.05$) in type 1 diabetes group during the experiment period Figure (4) (1.98 ±0.73 µmol/L) as compared with control group.

These results supported by the studies of Fatima *et al.* (2016) [15] and Domínguez *et al* (1998) [26] , they found significant decrease of blood serum glutathione (GSH) in type 1 diabetic patients in comparison with control group.

In diabetes mellitus, the increased blood glucose levels induce oxidative stress and decrease antioxidant defenses; oxidative stress may be amplified by a continuing cycle of metabolic stress, tissue damage and cell death, leading to increased free radical production and compromised free radical scavenger system; thus lower GSH levels may be due to the increased consumption of GSH in fighting free radicals for preventing oxidative damage in type 1 diabetic patients [27 , 28].

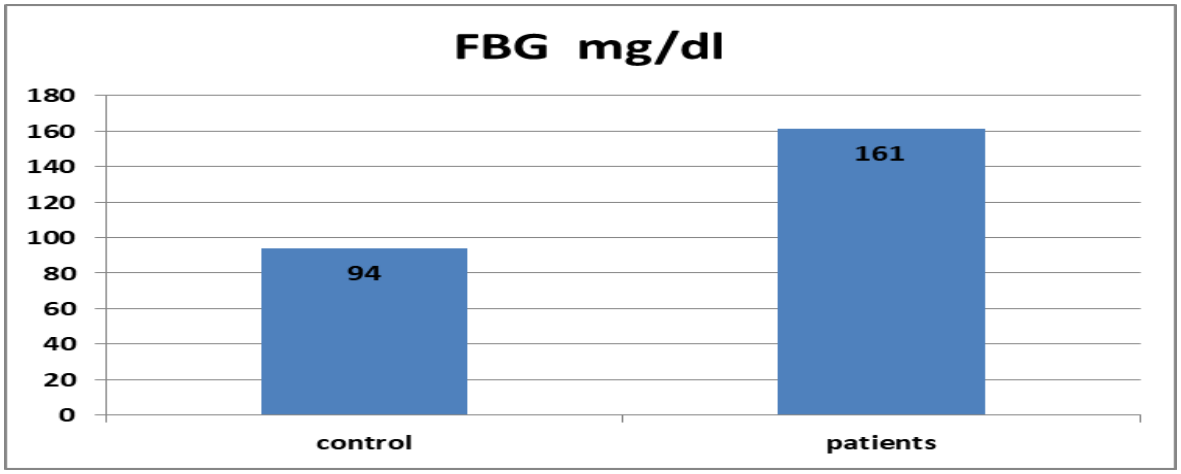

Figure (1): Concentrations of Fasting Blood Glucose (mg/dl) in the study groups.

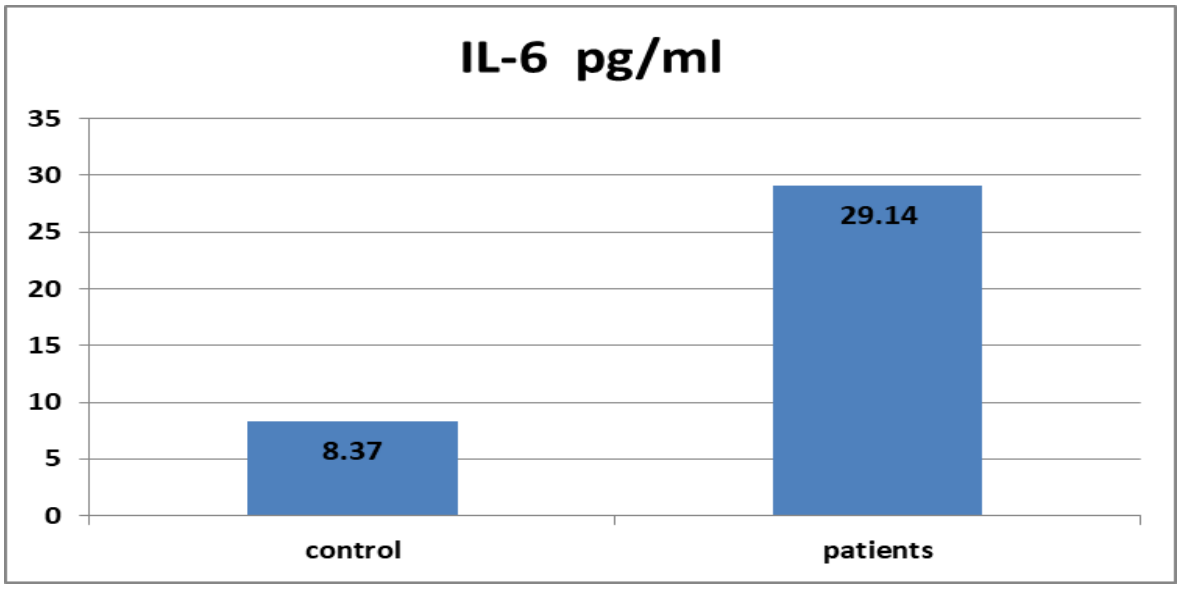

Figure 2: Concentrations of blood serum IL-6 (pg/ml) in the study groups

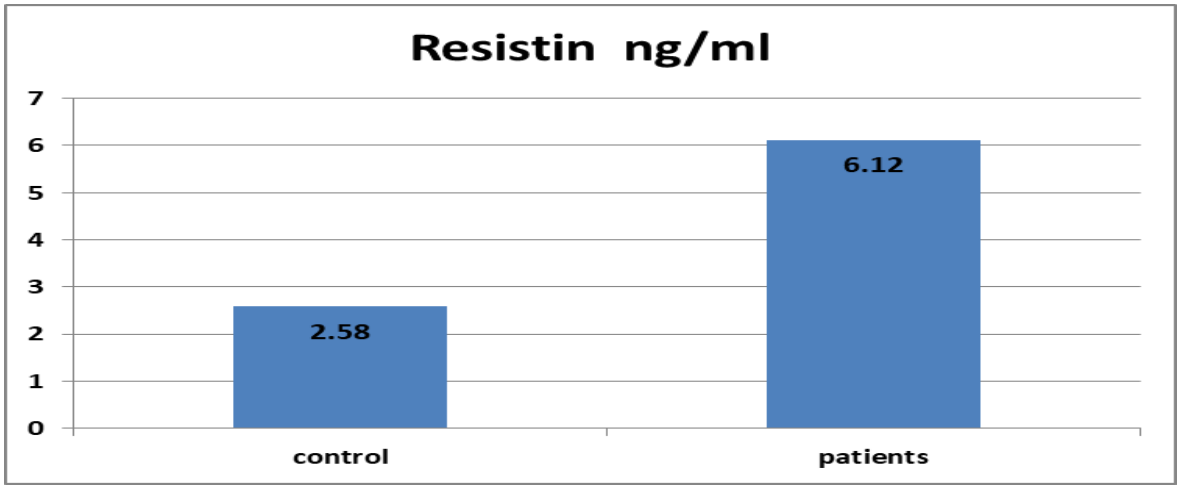

Figure 3: Concentrations of blood serum Resistin (ng/ml) in the study groups

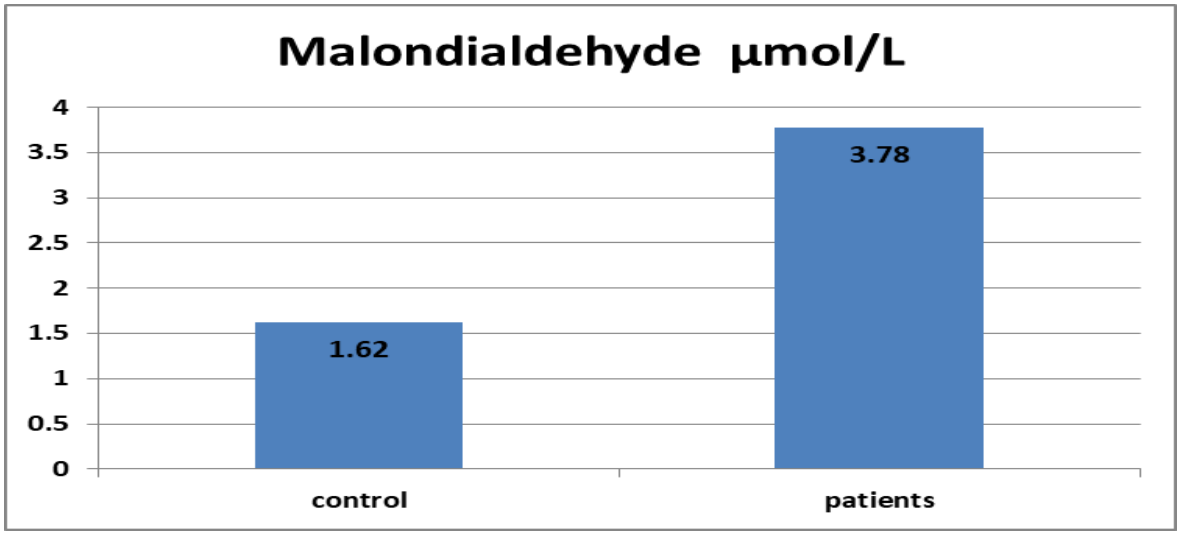

Figure 4: Concentrations of blood serum MDA (µmol / L) in the study groups.

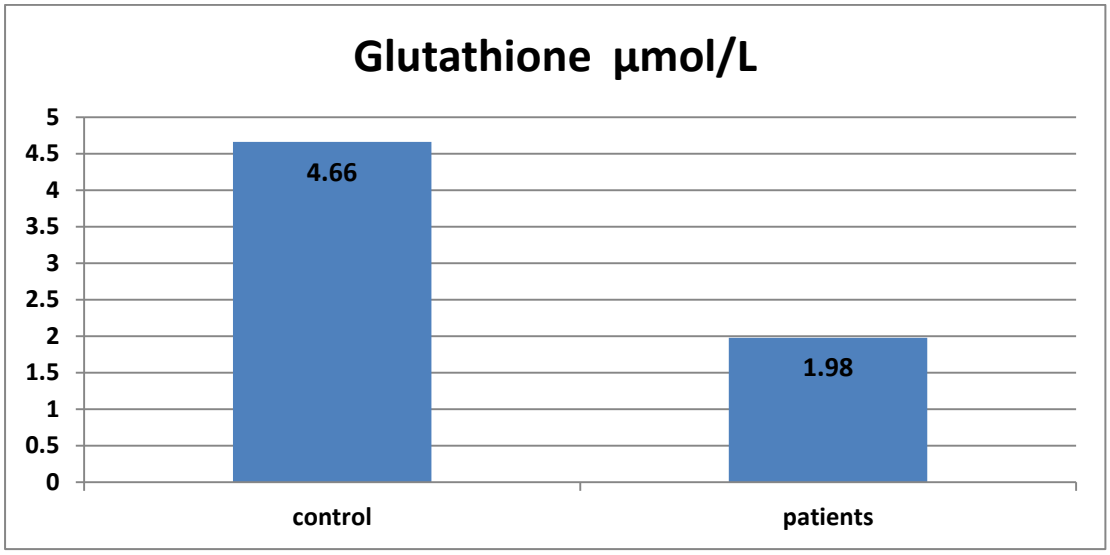

Figure 5: Concentrations of blood serum GSH (µmol/L) in the study groups

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