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Effect of Interleukin6 (IL-6) and Tumor Necrosis Factor (TNF-A) on Type 2 Diabetes Patients in Kirkuk City

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Annotation: This study was conducted on patients with type 2 diabetes in Kirkuk city. The study was conducted from October 2023 to mid-January 2024. 90 blood samples were collected from patients with diabetes. Blood samples were taken after fasting for at least 6 hours. Information about each case was recorded based on a pre-prepared form that included information related to the research topic. The samples were distributed into two groups: the first group included 25 blood samples from infected males and 25 blood samples from infected females. The second group included 20 blood samples from healthy males and 20 blood samples from healthy females. The ages of the samples ranged between 25-50 years. The samples were examined in private laboratories (Popular Work Laboratory and Hajjaj Laboratory) in Kirkuk. The tests were completed in the central laboratory at the College of Pharmacy at Tikrit University. The results of the immune variables showed no significant differences in the concentration of interleukin 6 (IL-6) and tumor necrosis factor (TNF-α) in the serum of diabetic patients

compared to the control group. The results of the immune variables showed no significant differences in the concentration of interleukin 6 (IL-6) and tumor necrosis factor (TNF-α) in the serum of diabetic patients, males and females, compared to the control group, males and females. The results of the immune variables showed no significant differences in the concentration of interleukin 6 (IL-6) and tumor necrosis factor (TNF-α) in the serum of diabetic patients, compared to the control group, in the three age groups. This study aimed to identify the effect of type 2 diabetes on some immune variables.

Keywords: interleukin-6, tumor necrosis factor, type 2 diabetes, insulin resistance.

1. Introduction

Diabetes Mellitus is a chronic disease that affects the individual's biological fitness. The World Health Organization (WHO) estimates that there are more than 171 million people with diabetes in 2000, and the number may reach 366 million in 2030, meaning that there is a continuous increase in the incidence of this disease. In Iraq, statistics for 2019 indicated that more than 13.9% of adults suffer from diabetes (Saeedi et al., 2019). Chronic hyperglycemia is a collective term for all metabolic disorders whose main result is either a disorder in insulin secretion or different degrees of insulin resistance (IR) or both (America Dental Association, 2018; Naz & Ahuja, 2022). The American Diabetes Association (ADA) has classified diabetes into four main groups: insulin-dependent diabetes mellitus (T1DM), non-insulin-dependent diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and other types of diabetes (ADA, 2018). T1DM, T2DM, and GDM are the most common types. T2DM is characterized by abnormally low insulin production or resistance (Rabbani et al., 2022). Cytokines are important molecules that regulate gene development and immune system function. They are small, normally secreted proteins that are produced upon activation of immune system cells, including lymphocytes and myeloid cells. Many cytokines have been described, and several are recognized as central players in immune responses and human disease (Lewis and Blutt, 2019). Cytokines are grouped into distinct families based on differences in amino acid sequence homology and structural features. These features include higher-order protein structure and the use of certain membrane-bound cytokine β-receptors for signal transduction, in particular the interleukin IL-6/IL-12 family (Belladonna and Grohmann, 2013). Increasing evidence suggests that a chronic low-grade inflammatory state is associated with T2DM. Low-grade inflammation is mediated by high levels of inflammatory cytokines such as IL-6. Some suggest that IL-6 may be important for maintaining glucose homeostasis (Matthews et al., 2010). It affects glucose metabolism and homeostasis in various organs in the body through direct and/or

indirect action (Kristiansen et al., 2005). Elevated levels of IL-6 are progressively associated with the development of T2DM. This suggests that IL-6 is also a major contributor to the induction of low-grade tissue inflammation, systemic inflammation, or both, and promotes the development of insulin resistance (Casazza et al., 2010) in T2DM. TNF-α is an adipose cytokine involved in systemic inflammation and induces the acute phase reaction. TNF-α is primarily produced by macrophages as well as adipocytes (Hachim et al., 2017). TNF-α inhibits insulin transport and disrupts glucose metabolism (Rains and Jain, 2011). Altered TNF-α metabolism is implicated in metabolic syndrome such as weight gain and insulin resistance, which explains why TNF-α metabolism disorders contribute to T2DM and disease progression (Swaroop et al., 2012). Tumor Necrosis Factor-α (TNF-α) is known to be a cellular kinetic, generated by macrophages, and is a key component of the immune system in eliminating infection by enhancing macrophage activity and nitric oxide (Singh et al., 2016). Adipocytes secrete several cytokines involved in insulin resistance and beta cell dysfunction including C-reactive protein (CRP), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and leptin. Populations at risk for developing type 2 diabetes are those who are obese or have higher concentrations of acute-phase inflammatory cytokines such as CRP, fibrinogen, IL-6, and TNFα (Reilly and Saltiel, 2017).

2. Materials and methods

2.1 Investigated Samples

90 blood samples were collected from donors, divided into 50 samples from patients with type 2 diabetes, 25 samples from females and 25 samples from males. 40 samples of healthy, normal, non-diabetic individuals, 20 samples from females and 20 samples from males, where the samples were collected from private laboratories in Amal Al-Shaabi and in the Hajjaj area in Kirkuk city for the period from 10/1/2023 to 1/15/2024, and the analyses were conducted in the central laboratories at the College of Pharmacy / Tikrit University. The age group of the samples was from (25- (50 years). The patient samples were divided according to the patient's gender, patient's age, body mass index, 20 control samples included healthy females, 20 control samples included healthy males. The level of IL-6 and the level of FNT-α were estimated based on the Enzyme Linked Immune Sorbent Assay (ELISA) technique.

3. Results and discussion

The results in Table (1) showed no significant differences at the level of (P 0.05), as we find no change in the concentration of IL-6 in diabetic patients (786 ± 36.1) (ng/L) compared to the control group (826 ± 60.6) (ng/L).

The results in Table (1) showed significant differences at the level of (P 0.05), as we find no change in the concentration of TNF-α in diabetic patients (1225 ± 586) (pg/mL) compared to the control group (1392.93 ± 1248.0) (pg/mL).

Table (1): Mean ± standard deviation of the results of the immune variables (TNF-α, IL-6) under study.

	Patients n=50	Control n=40	P-Value
IL-6 (mg/dL) Mean±Std	786 ± 36.1	826 ± 60.6	0.712 ns
TNF-α (mg/dL) Mean±Std	1225 ± 586	1248.0± 1392.93	0.879 ns

The results in Table (2) indicated a variation in IL-6 levels according to gender among the studied groups. The value of the significant difference was (P 0.05), as no significant difference was

recorded in the level of IL-6 between male patients (832.8 ± 44.4) compared to female patients (836.6 ± 48.2). The results of the study were also in the healthy group, as we did not record a significant difference in the level of IL-6 in male control (863.0 ± 53.2) compared to female control (859.3 ± 42.2). The results in Table (2) indicated a variation in TNF-α levels according to gender among the studied groups. The value of the significant difference was (P 0.05), as no significant difference was recorded in the level of TNF-α between male patients (1307 ± 51.7) compared to female patients (1318 ± 64.1) The results of the study were also in the healthy group, as we did not record a significant difference in the level of TNF-α in control males (1337 ± 81.2) compared to control females (1304 ± 79.6).

Table (2): Mean ± standard deviation of the results of immune variables (IL-6, TNF-α) according to gender.

	Patients		**Control**		**P-Value**
	n= 25 Males	**n= 25 Females**	**n= 20 Males**	**n= 20 Females**	
IL-6 (mg/dL) Mean±Std	832.8 ± 44.4 a	836.6 ± 48.2 a	863.0 ± 53.2 a	859.3 ± 42.2 a	0.819 ns
TNF-α (mg/dL) Mean±Std	1307 ± 51.7 a	1318 ± 64.1 a	1337 ± 81.2 a	1304 ± 79.6 a	0.882 ns

The results in Table (3) indicated a variation in IL-6 levels according to age Among the studied groups, the value of the significant difference was (P) 0.05, as no significant difference was recorded between the age group.

The first age group (25-35) years for patients (811.0 ± 71.6) and control (842.0 ± 54.3)

The second group (45-36) years for patients (804.3 ± 59.2) and control (845.0 ± 59.0)

The third group (46-50) years for patients (808.1 ± 47.1) and control (830.0 ± 52.4)

The results in Table (3) indicated a variation in TNF-α levels according to age among the studied groups, and the value of the significant difference was (P) 0.05, as no significant difference was recorded between the age group.

The first age group (25-35) years for patients (1334 ± 78.8) And control (1346 ± 31.0)

The second age category (36-45) years for patients (1331 ± 48.3) and control (1351 ± 76.8)

The third age category (50-46) years for patients (1324 ± 58.6) and control (1355 ± 52.7)

Table (3): Mean ± standard deviation of the results of immune variables (IL-6, TNF-α) according to age.

	The first category		**The second category**		**The third category**		**P-Value**
	n= 12 Infected	**n= 21 Control**	**n= 22 Infected**	**n= 13 Control**	**n= 16 Infected**	**n= 6 Control**	
IL-6 (mg/dL) Mean±Std	811.0 ±71.6 a	842.0 ±54.3 a	804.3 ±59.2 a	845.0 ±59.0 a	808.1 ±47.1 a	830.0 ±52.4 a	0.963 ns
TNF-α (mg/dL) Mean±Std	1334 ±78.8 a	1346 ±31.0 a	1331 ±48.3 a	1351 ±76.8 a	1324 ±58.6 a	1355 ±52.7 a	0.996 ns

Discussion:

The results of our study showed no significant difference in IL-6 and TNF-α between the infected and control study groups. The reason for these results is that these factors increase in the presence

of inflammatory factors, which is evidence that the studied groups have low inflammatory rates, in addition to the BMI being within the normal range.

The results of our study did not agree with the study conducted by El-Mikkawy et al. (2020), who proved that the increase in IL-6 occurs due to the chronic inflammatory state resulting from a disorder in metabolism and energy production, which leads to a change in body weight and the change may cause diseases including: atherosclerosis, blood vessels, heart disease, and others, i.e.: there is a close relationship between metabolic pathways and inflammation.

IL-6 is an inflammatory cytokine that plays a crucial role in the development of IR and T2DM (Bastard et al 2006). It affects glucose metabolism and homeostasis in various organs through direct or indirect action or both, which suggests that IL-6 participates with pro-inflammatory factors in the development and occurrence of IR and T2 DM (Kristiansen et al., 2005).

Tumor necrosis factor (FNT-α) is a mediator of obesity-related IR and atherosclerosis, and it has been suggested that high blood glucose affects oxidative stress levels and oxidative stress also increases TNF-α activity (Esposito et al., 2002). IL-6 and TNF-α are major factors that induce insulin resistance by inducing various inflammatory responses (Rehman et al., 2016). Other studies have shown that IL-6 and TNF-α promote lipolysis, inhibit lipid synthesis and lower blood lipids, so plasma levels of IL-6 and TNF-α are significantly increased in patients with obesity or osteoarthritis and also act as regulators of articular cartilage (Wang & He, 2018). Inflammation is supported by increased expression of pro-inflammatory cytokines secreted by macrophages in diabetic tissues. Chronic inflammation is characterized by persistent infiltration of immune cells and secreted pro-inflammatory cytokines that delay tissue healing in DM patients (Littig et al., 2022). Adequate expression of IL-6 can accelerate wound healing, while overexpression can inhibit wound healing (Gulo & Novida, 2022). The main components of the immune network associated with IL-6 are phase proteins, the main stimulus for their production in hepatocytes is IL-6. T2DM has been identified as an immune-mediated disease that leads to impaired insulin signaling and selective destruction of insulin-producing β cells, in which cytokines play an important role (Al-Salih & Ali, 2021). People with chronic kidney disease (CKD) have higher levels of the inflammatory biomarkers CRP and TNF-α, and high blood glucose levels in type 2 diabetes patients can affect the concentrations of inflammatory biomarkers (Yeo, E., et al., 2010). Several findings have suggested that diabetes control improves and maintains the immune response capacity, which reduces susceptibility to infection.

The results of our study showed that there was no significant difference in the levels of IL-6 and TNF-α in males and females in the case of infected and healthy people, as IL-6 and TNF-α are inflammatory indicators and the gender factor does not have a significant effect on them, in addition to the fact that most of the studied samples were among diabetic patients who controlled the disease, and many results indicated that controlling diabetes led to improving and maintaining the immune response capacity, which reduces exposure to infection. These results were consistent with what was stated by Jaganathan et al. (2018), which indicates an increase in inflammatory activity with a decrease in the risk of developing type 2 diabetes, and the reason for its decrease in patients may be due to a response to diabetes damage and varies according to the metabolic state and cellular infection. The results of our study are consistent with studies by Yushan Li et al. (2022) which showed that gender did not affect the level of inflammatory factors and leptin levels in T2DM. Although this study did not specifically investigate IL-6, it provides insight into the effect of gender on some biomarkers in diabetic patients. These results are not consistent with Gatea (2022) who found a nonlinear increase in the response to this cytokine in females compared to males and attributed this to the response to acute psychological and physical stress among women. The observed gender difference in inflammatory response may be related to evolutionary factors. Females have evolved to serve some separate social roles that require a greater immune response, and have evolved a stronger immune system, allowing for more efficient protection from infection. In fact, females show a greater immune response to antigenic challenges including infection, injury, and vaccination. This evolutionary protective immune response in women is balanced by facilitating

autoimmune damage. Indeed, autoimmune disorders are more prevalent in women (Hussain et al., 2022). We believe that this is because gender is an important biological factor that potentially modulates IL-6 reactivity in response to pain, a psychophysical stressor characterized by an unpleasant sensory experience. Chronic inflammation is supported by increased expression of pro-inflammatory cytokines, which are secreted by macrophages in DFU tissues, and these findings support that chronic inflammation mediated by infiltrating immune cells and persistent secreted pro-inflammatory cytokines mediates poor healing in DFUs (Littig et al., 2022). T2DM is a pro-inflammatory metabolic disorder characterized by hyperglycemia and increased levels of circulating cytokines, suggesting a causal role for inflammation in its etiology (Hussain et al., 2022).

The results of our study showed that there was no significant difference in IL-6 and TNF-α in all age groups. These results contradicted what Pickup and his group (1997) showed that the concentration of tumor necrosis factor was higher in diabetic patients compared to the control group. Our study also did not agree with what Liu and his group (2016) showed that tumor necrosis factor levels increased with age in healthy people. We believe that the reason for this is that high levels of inflammatory cytokines can predict the onset and development of type 2 diabetes in the future and also provide insight into the potential clinical benefit of some inflammatory factors as biomarkers for early detection of type 2 diabetes.

4. Conclusions

There was no significant difference in the concentration of interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) in people with type 2 diabetes compared to healthy people, as well as when studying this hormone in terms of the patient's age and gender.

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