



## RESEARCH ARTICLE

## Evaluation of Some Inflammatory Cytokines Levels as A Marker for Diabetes

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### Abstract

From the different causes of death diabetes has an advanced rank in the first 10. So the aim of this study was to relate between some pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and TGF- $\beta$ 1) and infection with diabetes as biomarkers for infection. Forty male Wister albino Streptozotocin (STZ) induced diabetic rats were used as a model for estimation of the related cytokines. The animals were divided in two groups, the first group was given a placebo, whereas the second was given Streptozotocin at a single dose of 70 mg/kg body weight to induce diabetes mellitus. Each rat's blood glucose level was measured 72 hours later. Diabetes was successfully induced in rats with blood glucose levels greater than 300 mg/dl. After 7 days, all groups were sacrificed and immunological responses, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin (IL-6), and transforming growth factor (TGF- $\beta$ 1) were measured. Glucose levels were shown to be significantly higher in the group that was given Streptozotocin at a single dose of 70 mg/kg body weight, with a percent change of 217.5% when compared to the control group. While in the diabetic group, there was also a large increase in IL-6, TNF- $\alpha$  and TGF- $\beta$ 1 levels, with a percent change of 82.24%, 77.7% and 74.5% respectively. From these results we can recommend these cytokines as a biomarkers for diabetes mellitus disease.

### Keywords

Diabetes, Cytokines, Biomarkers

### Introduction

Diabetes mellitus (DM) is a serious problem prevailed all over the world in low-, middle- and high-income countries. It is considered as a pool of diverse metabolic disorders both type 1 and type 2, diabetes comprise abnormalities of insulin action, which includes

insulin insensitivity and resistance characterized by hyper-glycemic episodes as a result to relative or absolute insulin deficiency which accompanied with high blood sugar levels and dysfunction of pancreas [1]. There are many reasons to focus on diabetes such as, it is considered as a developing epidemic because the elevation in global deaths about 70% since 2000 now it is the 9<sup>th</sup> leading cause of death in world. Rise in premature deaths (5% increase since 2010). Rise in total numbers of diagnosed diabetic people 420 million today, 570 million (2030) and 700 million (2045). Diagnosed diabetic people are of great danger to be ill or die as a result of COVID-19 infection. Also lack of diagnosis and treatment, 4/5 persons with undiagnosed diabetes are supposed to suffer from heart problems, strokes and amputations WHO [2]. DM is accompanying with long-term problems like retinopathy, nephropathy, neuropathy and angiopathy Seedeви, et al. [3]. Furthermore, DM is considered as a main threat for cardiovascular disorders, namely, ischaemic heart disease, cerebral stroke and peripheral artery disease, leading to elevation of death rate between diabetic patients (2015) [4].

Mutie, et al. (2017) [5] suggested that using of biomarkers as a precision medicine for detecting and prevention of diabetes is a targeted therapeutic strategy. Although precision diabetes medicine is confined to pharmacotherapy, using biomarkers to personalize lifestyle recommendations, in type 2 diabetes mellitus (T2DM) treatment is becoming a real possibility Franks and Poveda [6]. Both adapted and precision medicine



**Citation:** Abo-Zaid MA, Mohammed SS, Ismail AH (2022) Evaluation of Some Inflammatory Cytokines Levels as A Marker for Diabetes. Int J Immunol Immunother 9:065. doi.org/10.23937/2378-3672/1410065

**Accepted:** June 27, 2022; **Published:** September 05, 2022

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tactics in type 2 diabetes mellitus should therefore be an important and urgent consideration, as many individuals with T2DM have multiple (cardiovascular) comorbidities McCarthy [7].

Cytokines are pleiotropic polypeptides that operate on cells to regulate inflammatory and immunological responses. They have a crucial role in the pathophysiology of a variety of illnesses, including diabetes. Diabetes triggers a number of pathogenic responses and microvascular consequences, including chronic inflammation and innate immune system stimulation. For example IL-1, IL-6, and IL-18, and TNF- $\alpha$ , composed a part of diabetic nephropathy progression and growth Navarro-Gonzalez and Mora-Fernandez [8].

As a result of the pandemic prevalence of diabetes and its risky side effects, it was a must to discover a new strategies to identify and even more successful treatment of diabetic patients. So the aim of this study concentrated on studying if the circulating biomarkers will be a good tracker for the disease progression as well as medication effectiveness or not.

## Materials and Methods

### Materials

1. Streptozotocin was purchased from (Sigma-Aldrich Inc., St. Louis, MO., USA).
2. 50 mM sodium citrate buffer (enzyme grade; Fisher), pH 4.5: Prepared immediately before use.

### Methods

**Induction of diabetes:** Induction of diabetes in the experimental animals was conducted as described by [9].

**Animals and experimental design:** Thirty male Wister rats, aged from 6 to 8 weeks and their weight were ranged from 130 to 150 gm. Rats were obtained from the Medical Research Centre at the Faculty of Medicine, Jazan, KSA. Rats were kept in cages under hygienic conditions with light/dark cycles of 12 h at 25°C, fed on standard rodent chow and supplied with water. The rats were divided into two groups, first group control group (n = 10), intraperitoneally injected with a single dose sodium citrate buffer solution the same volume as the other group. Second group (n = 20) was injected intraperitoneally with Streptozotocin as described by Ikebukuro, et al. [10] with a single dose of (Streptozotocin) at 70 mg/kg body weight. All the experiments were performed in agreement with the regulations of the institutional animal ethics committee of Jazan University, Jazan, Kingdom of Saudi Arabia.

**Collection of blood sample:** All animals were sacrificed under light ether anesthesia after 7 days and blood samples were collected by cardiac puncture in a syringe. Immediately after sacrifice, blood was collected and divided into 2 portions, the first portion was

collected in Sodium Fluoride tubes for blood glucose analysis, and the second portion was collected with plane tube for the separation of serum by centrifugation at 3000 r.p.m for 15 min and then stored at -20 °C until used for immunological analysis.

**Determination of blood glucose level:** Blood was collected at the beginning of the experiment retro-orbitally from the inner canthus of the eye using micro hematocrit capillaries in oxalate-sodium fluoride in Eppendorf tubes. Glucose was estimated at the beginning and end of the experiment by glucose oxidase method using the kit from Randox, Cairo, Egypt.

**Assay of cytokines:** The levels of TGF- $\beta$ 1, TNF- $\alpha$  and IL-6 were measured by ELISA and according to the method adopted by Whiteside [11] using the kits following the manufacturer's instructions (Endogen Company, USA).

**Statistical analysis:** The results obtained in the present study were expressed as mean  $\pm$  SE and statistical analysis were performed using SPSS (version 19). The statistical differences between various groups were analyzed by the student's t-test and the significance was observed at the p < 0.01 and p < 0.001 levels.

## Results

### Blood glucose level

As shown in Table 1 blood glucose level in rats after induction of diabetes with Streptozotocin after 3 days at dose of 70 mg/kg of body weight revealed high significant increase with percent change 217.5% (p < 0.001) incomparison with control value. The data obtained in Table 2 shown high significant increase in

**Table 1:** Effect of Streptozotocin administered to rats at 70 mg/kg body weight on blood glucose level in rats compared to control group after 3 days.

Parameters % of change	Control (Non-diabetic)	Streptozotocin 70 mg/kg body weight (Diabetic rats)
Glucose level mg/dl	110.4 $\pm$ 2.5	350.6 $\pm$ 5.1***
% of change		217.5%

\*\*\* Significantly different from control at p < 0.001

**Table 2:** Effect of Streptozotocin administered to rats at 70 mg/kg body weight on blood glucose level in rats compared to control group after 7 days.

Parameters % of change	Control (Non-diabetic)	Streptozotocin 70 mg/kg body weight (Diabetic rats)
Glucose level mg/dl	125.58 $\pm$ 2.5	400.74 $\pm$ 5.1***
% of change		219.1%

\*\*\* Significantly different from control at p < 0.001

**Table 3:** Level of cytokines IL-6, TNF- $\alpha$  and TGF- $\beta$ 1 in rats after induction of diabetic with Streptozotocin compared to control group.

Parameters	Control (Non-diabetic)	Streptozotocin 70 mg/kg body weight (Diabetic rats)
IL-6 (pg/ml)	230.64 $\pm$ 5.2	420.34 $\pm$ 6.2***
% of change		82.24%
TNF- $\alpha$ (pg/ml)	180.24 $\pm$ 2.5	320.45 $\pm$ 3.3***
% of change		77.7%
TGF- $\beta$ 1 (pg/ml)	160.52 $\pm$ 2.8	280.63 $\pm$ 4.1***
% of change		74.50%

\*\*\*Significantly different from control at  $p < 0.001$ .

blood glucose level in rats after induction of diabetic with Streptozotocin 7 days (G2) amounted to 219.1% ( $p < 0.001$ ) as compared to control group.

### Level of cytokines IL-6

The data obtained in Table 3 indicated high significant elevation in serum IL-6 in rats after induction of diabetes with Streptozotocin at dose of 70 mg/kg of body weight with percent change 82.24% ( $p < 0.001$ ) in comparison with the control group.

### Level of cytokines TNF- $\alpha$

As shown in Table 3 Level of cytokines TNF- $\alpha$  in rats after induction of diabetic with Streptozotocin at dose 70 mg/kg of body weight revealed high significant increase with percent change (77.7%) in comparison with control value.

### Level of cytokines TGF- $\beta$ 1

Table 3 show high significant increase in the level of cytokines TGF- $\beta$ 1 in rats after induction of diabetic with Streptozotocin at dose 70 mg/kg of body weight with percent change 74.5% ( $p < 0.001$ ) in comparison with control value.

## Discussion

The goal of this study was to determine the levels of TGF- $\beta$ 1, IL-6, and TNF- $\alpha$  in diabetic group. Using the Streptozotocin-induced diabetic rat model indicates that the diabetic condition is accompanying with a generalized induction of cytokines release. Immediately, release of pro-/anti-inflammatory cytokines and some growth factors such as IL-6, IL-10 and TNF-alpha from Müller cells were considerably enhanced in response to hyperglycemia as result for stimulated Streptozotocin-induced diabetic rat model Coughlin, et al. [12].

As a result, diabetic rat models were crucial in understanding the pathophysiology of human diabetes and associated consequences such retinopathy, neuropathy, and nephropathy Al-Awar, et al. [13]. In the current study, the serum glucose level recorded highly significant increase of 217.5% in rat-diabetic

group induced with Streptozotocin (STZ) at dose 70 mg/kg body weight. These findings are consistent with those of [3,14] who found Streptozotocin to be one of the most often utilized drugs to induce diabetes in rats, resulting in a considerable increase in plasma glucose levels. Previous studies revealed that Streptozotocin induced deterioration of pancreatic cells may be due to oxidative stress, as a result, Streptozotocin inhibits cellular reproduction with a far lower dose than is required to inhibit the substrate link to DNA or many of the enzymes involved in DNA synthesis Wu and Yan [15]. Although, Streptozotocin stops cells from entering mitosis, no one phase of the biological cycle is particularly vulnerable to its fatal effects Nagarchi, et al. [16]. The current study's findings revealed that serum cytokine IL-6 level were significantly higher in diabetic group 82.24%  $p < 0.001$  in compared to control group. These findings are consistent with Genovese, et al. [17] who stated that high levels of IL-6 are a risk factor for T2D and that IL-6 alone or in combination with IL-1 $\beta$ , impairs  $\beta$  cell activity. Furthermore, IL-15 and IL-6 could be employed as biomarkers for the development risk of autoimmune diabetes Siewko, et al. [18]. In addition, the outcomes are in line with Robinson, et al. [19] who mentioned that IL-6 is a pleiotropic cytokine that can be found in high levels in the blood and vitreous of DR (diabetic retinopathy) patients. Jorns, et al. [20] who reported that during the inflammation process in the T1D pancreas, the pro-inflammatory cytokine IL-6 was expressed in all immune cell subtypes. Tsalamandris, et al. [21] indicated that IL-6 plays a role in pathogenesis of diabetic. The buildup of pro-inflammatory cytokines TGF- $\beta$ 1, IL-6, IL-23 and TNF-alpha in the plasma and tissues of diabetic patients suggests a role in the development of diabetic complications. In fact, the short-duration diabetic model may have demonstrated reversibility of diabetes-induced pro-inflammatory cytokines due to the interaction between monocytes and glucose molecules Zhang, et al. [22]. The current study also demonstrates a significant increase in serum TNF- $\alpha$  in diabetic rats with percent of change 77.7%. These results confirm the result of Graneri, et al. [23] who found that tumor necrosis factor (TNF- $\alpha$ ) production is increased under acute or chronic hyperglycemia in diabetic animals. Akash, et al. [24] stated that the first pro-inflammatory cytokine recognized for its involvement in pathogenesis of insulin resistance and T2DM was TNF- $\alpha$ .

Tumor necrosis factor - alpha (TNF- $\alpha$ ) has been shown to lower the expression of the insulin-regulated glucose transporter type 4 (GLUT4), which is found mostly in adipocytes, skeletal, and cardiac muscles. Elevations in TNF- $\alpha$  and IL-1 $\beta$  have similar effects on glucose metabolism, insulin resistance, pancreatic cell function, and diabetes risk Urman, et al. [25]. TNF- $\alpha$  is a pro-inflammatory cytokine that triggers secretion of other cytokines such as IL-6 and IL-1 $\beta$ . Likewise, the role of pro-inflammatory cytokines in disrupting

insulin signaling pathway could happen at various levels Husna, et al. [26]. Also, TNF- $\alpha$  was found to be significantly related with diabetes, according to Mirza, et al. [27], and was most significantly raised in the group of individuals with glycated hemoglobin (HbA1c) values more than 6.5%. Previous data indicated that TNF-alpha plasma levels are increased in type 1 diabetes mellitus and reveal a significant association with metabolic long-term control parameters, HbA1c and fructosamine for glycaemic control, and HDL cholesterol for triglyceride metabolism, as well with lipid peroxidation. TNF- $\alpha$  is associated with concurrent obesity and T2DM and correlates with HbA1c [14,28,29] reported that that two recent meta-analyses indicated that both patients with type 1 and type 2 diabetes mellitus have significantly elevated levels of serum TNF- $\alpha$  which showed a positive correlation with insulin resistance.

Liu, et al. [30] indicated that increased levels of inflammatory cytokines, such as TNF-alpha, were found to be significantly linked to an increased risk of T2DM. Furthermore, type 1 diabetes (T1D) is a T cell-mediated autoimmune disease characterized by the expression and release of pro-inflammatory cytokines, particularly tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin 1 beta (IL-1 $\beta$ ), as well as other mediators, by pancreatic islet infiltrating immune cells, resulting in selective apoptotic cell death Atkinson, et al. [31].

In the present study, it was found that the expression TGF- $\beta$ 1 is one of good player in the pro-inflammatory cytokines system. The present data revealed that percent change in TGF- $\beta$ 1 reached up to 74.5% in the diabetic group. These findings confirm the results of Lechleitner, et al. [32] who reported that increased levels of TGF- 1 $\beta$  may contribute to pathophysiology of diabetes mellitus in the long term. TGF- 1 $\beta$  is an important cytokine for the improvement and progression of renal and endothelial of complications in diabetes.

Previous studies have explained the correlation between of hyperglycemia and increasing TGF- $\beta$ 1 expression through different pathways Hydarpoor, et al. [33]. Additionally, TGF- $\beta$ 1 plays a crucial role in the immune system. It is involved with differentiation, chemotaxis, survival, and lymphocyte proliferation. Regulation of leukocyte function is an important role played by TGF-1 $\beta$ , and dysregulation can cause autoimmune diseases, for example type T1DM. In T1DM, the destruction of insulin-producing  $\beta$  cells takes place and is mediated by T cells, important targets of TGF- $\beta$ 1. Blocking of TGF- $\beta$ 1 signaling in mice has led to an autoimmune phenotype, involving activation and differentiation of T cells. In  $\beta$  cells, TGF- $\beta$ 1 is expressed under the influence of insulin promoters and inhibits T1DM from developing Lee, et al. [34]. Dysregulation of TGF- $\beta$ 1 pathway is specially associated with progression of various complications associated with DM, such as diabetic neuropathy, and delayed wound healing. Features of diabetic neuropathy include glomerular

sclerosis, tubulointerstitial fibrosis, extracellular matrix (ECM) alterations, and mesangial expansion. TGF- $\beta$ 1 is an important regulator of fibrosis associated with diabetic nephropathy as indicated by increased renal TGF- $\beta$ 1 expression in persistent hyperglycemic conditions of human patients and animal models of diabetes Wu, et al. [35]. Likewise, according to Braga Gomes, et al. [36] stated that TGF- $\beta$ 1 is a classically anti-inflammatory immune mediator, but it has been shown that in the presence of IL-6, which increases before the onset of T2D, TGF- $\beta$ 1 favors the differentiation of T helper 17 (Th17) cells, which are activated in many pro-inflammatory conditions. According to Hydarpoor, et al. [33], increased levels of pro-inflammatory cytokines released by peripheral blood mononuclear cells (PBMCs) are linked to atherosclerotic damage in T2DM, resulting in insulin resistance and  $\beta$  cell abnormalities. Previous studies revealed that the measurement of TGF- $\beta$ 1 serum levels in children and adolescents revealed a positive relationship between the duration of T1DM and complications in the vascular system Zorena, et al. [37]. Also, According to El-Sherbini, et al. [38], hyperglycemia is related with activation of glucose transport-1, which leads to TGF- $\beta$ 1 overexpression by mesangial tubular cells or infiltrating renal cells in individuals with T2D.

## Conclusion

The present results proved the relationship between high blood glucose levels and pro-inflammatory cytokines. Which occurs in the elevation of pro- IL-6, TNF- $\alpha$  and TGF- $\beta$ 1 concentrations in the diabetic rats. The regulations with prophylactic hyperglycemia and diabetes mellitus must be through the modulations of pro-inflammatory cytokines and the immune response against diabetes. So we can recommend these pro-inflammatory cytokines estimations as indicators for diabetes mellitus disease.

## Author Contributions

Mabrouk A. Abo-Zaid, Soha S. Mohammed and Ahmed H. Ismail researched data and contributed to discussion, wrote the manuscript draft and reviewed the final manuscript and followed publication process.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Funding Information

This manuscript is not funded.

## Acknowledgement

The facilities provided by the Faculty of Science at Jazan University, Saudi Arabia are greatly acknowledged and appreciated.

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