

# The Role Of The Biomarkers Interleukin-18 And Transforming Growth Factor-Beta-1 In Predicting And Detecting Diabetic Nephropathy In Patients With Type 2 Diabetes

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## Abstract

The current study was conducted on men suffering from type 2 diabetes in Dhi Qar Governorate, Iraq. The study aimed to assess the levels of some biomarkers in type 2 diabetic patients and their relationship to diabetic nephropathy, including interleukin18 and transforming growth factor beta1, and included in the study (88) blood samples from men only. They were distributed as follows: 60 serum samples from men with T2DM and 28 serum samples from healthy men. The study samples were collected in cooperation with Shatra Hospital, as samples were classified into patients according to the disease severity and the duration of . The results of the current study showed a significant increase in the concentration of IL-18 and TGFb-1 in the samples of patients suffering from T2DM compared with the healthy ones. This study concluded that there was a significant increase in the concentration of IL-18 in patients in the second category compared to the first category with respect to the disease severity , as well as a significant increase in the duration of disease in the first category compared with the second category, and this study concluded the possibility of adopting these indicators as early risk predictors of diabetic nephropathy.

**Key words:** Diabetes mellitus, Interleukin-18, Transforming growth factor beta 1, diabetic nephropathy, Biomarkers.

## INTRODUCTION

Diabetes Mellitus is a common metabolic disorder. It is estimated that 1 in 11 adults has diabetes worldwide and is responsible for 11% of deaths annually at a cost of \$760 billion (Zheng, Ley, & Hu, 2018). Time has been a rapidly spreading global concern (DeFronzo et al., 2015) and is associated with many risk factors, including genetics and lifestyle influences, but the most common risk factor is obesity, and a diet is usually recommended for diabetic patients (Evert et al., 2019). There are many dangerous complications of diabetes, including diabetic nephropathy, which is the most prevalent kidney disease and the main cause of kidney disease in adults (Collins et al., 2006). A chronic disease characterized by glomerular hypertrophy, proteinuria, decreased glomerular filtration, and renal fibrosis with loss of kidney function due to high glucose in the final stages of the development of diabetic nephropathy (Sun, Su, Li, & Wang, 2013).

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Interleukins are a group of protein compounds that belong to a type of cytokine produced by many cells in the body, including immune cells, and interleukins participate in many important cellular processes, including reproduction, maturation, migration, and adhesion (Ferreira, Borba, Bonetti, Leonart, & Pontarolo, 2018; Kata, Alsulaiti, & Adlan, 2022). It has an impact on the development of diseases such as autoimmune diseases, diabetes, tumor disorders, neurological diseases, as well as kidney diseases (Al-Akabi, Kata, & Khosho, 2019; Lissoni et al., 2020).

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The assessment of IL-18 levels in the human body can be used as an indicator for the development of many diseases, including kidney disease (Gu et al., 2021; Tabata, Sugiyama, Otsuki, & Kondo, 2020). The cytokine is involved in the development of diabetic nephropathy and the increased expression of IL-18 in DN is higher in renal tubular cells (Donate-Correa, Martín-Núñez, Muros-de-Fuentes, Mora-Fernández, & Navarro-González, 2015). High levels of IL-18 in plasma and urine were associated with diabetic nephropathy and it was noted that IL-18 is a prognostic marker for the development of DN in diabetic patients (Araki et al., 2007), where IL-18 levels in the blood and urinary tract are correlated to a large degree Urinary albumin (Nakamura et al., 2005) and that high levels of IL-18 in serum and urine in DN patients is one of the risk factors for the development of DN in the kidney tissues of DN patients, as IL-18 is excessively secreted in tubular epithelial cells (Miyachi, Takiyama, Honjyo, Tateno, & Haneda, 2009).

Transforming growth factor-beta was first identified as a protein secreted by carcinoma sarcoma cells (Harold L. Moses, Branum, Proper, & Robinson, 1981), and it was named transforming growth factor because of its ability to induce transformed tumour cells in normal cells. It has a variety of cellular functions not related to cellular transformation (H. L. Moses, 1985), and it is an extracellular protein that is mostly produced by a group of T cells (Kehrl et al., 1986) and has been found to be ubiquitously expressed by all cells (Sporn, Roberts, Wakefield, & Assoian, 1986) TGF- $\beta$ 1 is the main cytokine in the pathogenesis of kidney inflammation and fibrosis (Meng, Nikolic-Paterson, & Lan, 2016).

Renal fibrosis, one of the main histological features of progressive diabetic nephropathy, has been associated with increased expression of TGF- $\beta$ 1 in the kidneys (Chen et al., 2003), while glomerular and tubular interstitial expression of TGF- $\beta$ 1 increases in DN (Yamamoto, Itonaga, Marunouchi, & Majima, 2005). There is a positive relationship between increased renal TGF- $\beta$ 1 production in patients with type 2 diabetes (Li, Liu, & Lu, 2019).

TGF- $\beta$ 1 plays a role in a variety of biological activities, including cell proliferation and differentiation, wound healing, tissue homeostasis, apoptosis, and angiogenesis. Alterations in TGF- $\beta$ 1 pathways can lead to human diseases such as connective tissue disorders, fibrosis, and cancer (Schmierer & Hill, 2007), TGF- $\beta$ 1 also regulates functions for the generation and response of many types of immune cells (Sanjabi, Oh, & Li, 2017). It controls immunity by inhibiting the generation and function of effector T cells and dendritic cells (DCs). TGF- $\beta$ 1 also controls the systemic system. Autoimmunity by inhibiting natural killer (NK) cells and regulating the complex behaviour of neutrophils and phages, thus forming a network of negative immune regulatory inputs (David & Massagué, 2018; Mullen & Wrana, 2017).

## Materials and Methods:

### The Patients Group

60 serum samples were obtained from men suffering from type 2 diabetes. As the samples were divided according to the severity of the disease based on HbA1c, 37 serum samples for men whose HbA1c ranged between 8–9.4, and 23 serum samples for men whose HbA1c ranged between 9.5–13.5. Also, the samples were divided according to the time period of the disease, 34 serum samples for men with a period of illness ranging from 5–10 years, and 26 serum samples for men with a period of illness ranging from 11–20. Samples were collected at the specialized diabetes centre in Dhi Qar Governorate.

### The Healthy Group

A total of 28 serum samples were obtained from healthy male donors after laboratory and clinical tests were performed by a specialized doctor to ensure that they did not have type 2 diabetes.

### Preparation Of Serum

5 ml of ulnar venous blood was withdrawn using a medical syringe, the blood was placed in a glass gel tube and left for 10 minutes, and the tube was placed in a centrifuge at 3500 rpm for 15 minutes to obtain the blood serum. It was obtained in 1 ml Eppendorf tubes and the samples were stored at -80°C in a frozen state until tests were performed

### Estimation Of The Concentration Of Biomarkers

The concentration of the biomarkers (interleukin-18, transforming growth factor beta-1) was estimated using the well-known immunoassay (enzyme-linked immunoassay) (ELISA) of the Sandwich type using an ELISA reader of Chinese

origin and the biomarker kits provided by the American company My Bio Source based on the leaflet attached to each indicator at wavelength of 450 nm.

## STATISTICAL ANALYSIS

The data were statistically analysed using the SPSS program and the T-test to compare between patient samples and those of healthy controls, as well as the comparison between patients according to disease severity and time period of disease at the probability level of  $P \leq 0.01$  and  $P \leq 0.05$ .

## RESULTS

The results of the current study showed a significant increase at the level of probability ( $P \leq 0.01$ ) in the concentration of interleukin-18 among patients with type 2 diabetes ( $68.79 \pm 16.82$  ng/ml) compared to its concentration in the healthy controls ( $53.08 \pm 12.52$  ng/ml). Results There was a significant increase at the probability level ( $P \leq 0.01$ ) in the concentration of interleukin-18 in the second group that had a cumulative sugar of 9.5-13 compared with the first group. As for the disease period, the results of the statistical analysis showed a significant increase in the concentration of interleukin-18 in the group The first is compared with the second category at the same level of probability in patients with type 2 diabetes, as shown in Table (1).

**Table 1.** The concentration of interleukin-18 in the blood serum

Variables	No.	Mean $\pm$ SD	P value	
Participants	T2DM	60	$68.79^* \pm 16.82$ ng / ml	0.000
	Healthy men	28	$53.08 \pm 12.52$ ng / ml	
Disease Severity	HbA1c (8 – 9.4)	37	$61.008 \pm 15.56$ ng / ml	0.01
	HbA1c (9.5 -13)	23	$73.85^a \pm 23.76$ ng / ml	
Disease duration	5 – 10 years	34	$77.11^a \pm 29.71$ ng / ml	0.000
	11 – 20 years	26	$51.30 \pm 14.22$ ng / ml	

There is a significant increase at the level of probability  $P \leq 0.01$  in diabetic patients compared with healthy controls. A significant increase at the probability level  $P \leq 0.01$  for disease severity and duration of illness.

The results shown in Table (2) showed a significant increase at the probability level ( $P \leq 0.01$ ) in the level of transforming growth factor beta 1 in men with type 2 diabetes ( $174.21 \pm 59.88$  ng/ml) compared to its level in healthy subjects ( $50.93 \pm 9.81$  ng/ml). The results of the statistical analysis also showed that there was no significant difference at the level of probability ( $P > 0.05$ ) for the severity of the disease and the time period of the disease.

**Table (2) Concentration of transforming growth factor beta-1 in serum**

Variables	No.	Mean $\pm$ SD	P value	
Participants	T2DM	60	$174.12^* \pm 59.88$ ng / ml	0.000
	Healthy men	28	$50.93 \pm 9.81$ ng / ml	
Disease Severity	HbA1c (8 – 9.4)	37	$148.69 \pm 40.58$ ng / ml	0.08
	HbA1c (9.5 – 13)	23	$171.54 \pm 59.82$ ng / ml	
Disease duration	5 - 10 years	34	$175.23 \pm 57.80$ ng / ml	0.8
	11 – 20 years	26	$172.66 \pm 79.69$ ng / ml	

There is a significant increase at the probability level of  $P \leq 0.01$  in diabetic patients compared with healthy subjects.

## DISCUSSION

The results of the current study showed a significant increase in the concentration of interleukin-18 in type 2 diabetes compared with its concentration in healthy controls, as this result was consistent with the findings of (Hirooka & Nozaki, 2021; Olufunsho, Peace, Olayinka, & Joy, 2021). The increase in the level of interleukin-18 in the plasma is due to the rise in blood sugar in people with impaired glucose tolerance and this elevation in the level of interleukin-18 is associated with higher triglyceride levels, insulin control, and the insulin resistance index (Esposito et al., 2002; Fischer, Perstrup, Berntsen, Eskildsen, & Pedersen, 2005), and the increase in the levels of interleukin-18 in patients with type 2 diabetes may be due to the increase in the gene expression of interleukin-18 due to the increase in the level of sugar, and

also the increase in the cumulative sugar percentage that impairs the function of the cell and thus leads to an increase in the concentration of interleukin-18 as a result of the effect of cumulative hyperglycaemia on tubular cells and small and large blood vessels (Esposito et al., 2003). Polymorphisms in the interleukin-18 gene, impaired insulin sensitivity, and increased risk of infection. Metabolic syndrome may lead to an increase in the levels of interleukin-18 in the blood serum (Presta et al., 2009).

Interleukin-18 is expressed in tubular epithelial cells, which may occur by activating the Mitogen-Activated Protein Kinase (MAPK) pathways resulting from transforming growth factor beta, and high levels of interleukin in the blood are associated with urinary albumin excretion. The risk of developing diabetic nephropathy because it is usually associated with the development of renal dysfunction (Miyachi et al., 2009; Nakamura et al., 2005). The oxidative stress caused by hyperglycaemia activates the nuclear factor NF- $\kappa$ B that increases the concentration of interleukin 18 Activator of inflammation through its production of pro-inflammatory cytokines, endothelial apoptosis and atherosclerosis (Esposito et al., 2002), as fluctuations in glucose levels in the fasting state during the night and the postprandial states during the day lead to high levels of oxidative stress and inflammation in patients with Type 2 diabetes, as these fluctuations increase the stimulation of adipocytes to produce interleukin-18, and thus glucose fluctuations are a cause of large vascular complications. JNK in patients with type 2 diabetes. The c-Jun NH<sub>2</sub>-Kinase (JNK) pathway is involved in oxidative stress and inflammation. A key step in this process is JNK activation (Gill, Tsung, & Billiar, 2010; Rizzo, Barbieri, Marfella, & Paolisso, 2012), activation of JNK causes phosphorylation of c-Jun which in turn activates the transcription factor activator protein-1 (AP-1), which controls the expression levels of a number of genes including interleukin-18. Thus, the JNK pathway increases interleukin-18 levels in macrophages exposed to high and volatile glucose concentrations (Davis, 2000; Kim, Im, Han, Kang, & Choi, 2000).

In addition, the results of the statistical analysis of this study showed a significant increase in the concentration of interleukin-18 in the second category of disease severity compared with the first category. High levels increase the severity of oxidative stress, and thus greater damage to the macrophages and endothelial cells, and this leads to an increase in the production of interleukin-18, and the concentration of interleukin-18 increases due to high levels of sugar in people with impaired glucose tolerance, and thus glucose stimulates the production of interleukin. 18 As a result of the damage caused by glucose in the endothelial and macrophage cells (Bruun, Stallknecht, Helge, & Richelsen, 2007), the results of this study also showed a significant increase in the concentration of interleukin-18 in the first group compared with the second group for the time period of the disease, as this study was consistent with what was found (Mabrouk et al., 2013), and the reason for this may be the high levels of triglycerides at the beginning of the infection due to the diet, meaning that the percentage of The lipids producing interleukin-18 at the beginning of the infection is higher than the second group during the disease period, and therefore the levels of interleukin-18 increase in the first group compared with the second group (Hivert et al., 2009), or autoimmunity may be a reason for the high levels of interleukin-18 at the beginning of the infection compared with the progression of the infection because the immunity decreases with the development of the disease (Boraschi & Dinarello, 2006).

Interleukin-18 is produced biologically inactive and stored in the cytosol of the kidneys. Once matured by Caspase-1, it causes severe autoimmune diseases, infections, metabolic diseases, small and macrovascular diseases, which is the basis for causing diabetic nephropathy. Therefore, IL-18 is a marker of Disease progression and prediction (Fujita et al., 2012), and it is usually associated with the development of renal dysfunction, and its rise in serum is a risk factor in type 2 diabetes patients, as it leads to diabetic nephropathy and is excessively produced in patients with diabetes. Tubular epithelial cells in the kidney tissues of diabetic nephropathy patients, which may occur by activating MAPK pathways caused by transforming growth factor-beta (Miyachi et al., 2009), and its high levels in plasma and urine were positively associated with T2DM. Diabetic kidney Therefore, interleukin-18 is a prognostic marker for the development of diabetic nephropathy in patients with type 2 diabetes (Araki et al., 2007).

The results of the statistical analysis in the current study showed a significant increase in the levels of transforming growth factor beta 1 among men with type 2 diabetes compared with its level in healthy men, as the results of this study were consistent with the findings of (Daria & Pertseva, 2019; Pertseva, Borysova, & Chub, 2019), which indicates the high levels of transforming growth factor beta 1 in diabetic patients, which may be attributed to the high levels of glucose and cumulative sugar in the blood, meaning that there is a positive correlation between transforming growth factor beta 1 with high glucose and cumulative sugar, as Elevated glucose stimulates and activates the synthesis of diacylglycerol, then leads to the activation of protein kinase, which increases the synthesis of transforming growth factor-beta-1 in mesenchymal cells and tubular cells (Jiang, Liu, & Kang, 2001). Renin-angiotensin and reactive oxidant species (ROS) stimulate the production of transforming growth factor beta-1 in the kidneys in mesenchymal cells or in tubular cells (Sharma & Ziyadeh, 1995), and that oxidative stress and the polyol pathway play a role in increasing and activating transforming growth factor beta-1 levels, as the polyol pathway consists of two enzymes, Aldose reductase (AR) and sorbitol dehydrogenase (SDH), which reduces the enzyme AR glucose. To sorbitol and sorbitol dehydrogenase, then convert sorbitol to fructose, and this process increases the proportion of NAD and NADH, which may lead to oxidative stress and activation of protein kinase, which increases the levels of transforming growth factor beta 1 (Ramana, 2011), and the elevation of angiotensin-2 in mesenchymal cells Diabetes-induced glomerular endothelial cells may cause elevated TGF- $\beta$ 1 levels by generating hyperglycaemic ROS or by protein kinase activation of MAPK-dependent pathways (Morales et al., 2012).

Elevated levels of TGF- $\beta$ 1 contribute to renal fibrosis by stimulating proximal tubular and endothelial differentiation (Pardali, Sanchez-Duffhues, Gomez-Puerto, & Ten Dijke, 2017), and TGF-1 has pro-inflammatory properties as it can be controlled by TGF-beta. 1 In autoimmune cells, there are serious pathological consequences. The recruitment of leukocytes and fibroblasts is achieved by activating the renal immune cells present in patients with diabetic

nephropathy. This recruitment stimulates the expression of proinflammatory cytokines and chemical reaction, which increases the infiltration of macrophages and monocytes and thus leads to a pathological impairment. Renal function (Lv, Booz, Wang, Fan, & Roman, 2018), TNF- $\beta$ 1 recruits macrophages by stimulating production of chemokines including Tumor necrosis factor-alpha (TNF- $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1), which maintains high levels of TGF- $\beta$ 1 and thus TGF- $\beta$ 1 releases other cytokines that cause TGF- $\beta$ 1 Inflammation such as interleukin-8 in the proximal tubular cells and thus leads to nephropathy (Qi et al., 2006), in addition, transforming growth factor beta 1 enhances autophagy, which leads to the removal of protein aggregates and damaged organelles to maintain cellular homeostasis. However, the continuous activation Autophagy in renal tubular epithelial cells causes tubular degeneration and enhances renal fibrosis (Livingston et al., 2016), and high levels of transforming growth factor beta 1 in renal tubules lead to accumulation of autophagy and stimulation of autophagy-related genes in nearby tubular cells. Transforming growth factor-beta-1-stimulated autophagy is a mechanism of tubular degeneration that leads to renal interstitial fibrosis, causing impaired renal function (Xu et al., 2012). It leads to a lack of white blood cells, thus promoting the process of progressive glomerulosclerosis, which leads to the deterioration of collagen at different stages of the development of diabetic nephropathy and also promotes renal fibrosis in a negative way. It directly stimulates the transformation of tubular epithelial cells into myofibroblasts (Ding & Choi, 2014). Important in the early detection of diabetic nephropathy (Meng et al., 2016) and the results did not show a significant difference in the concentration of transforming growth factor beta in relation to the disease severity and the duration among patients with type 2 diabetes, and this study was consistent with the findings (Okada et al., 2007).

## CONCLUSION

The significant increase in the level of biomarkers in the current study may be one of the induced causes of diabetic nephropathy in patients with type II diabetes, therefore its adoption as risk indicators for early prediction of the occurrence of diabetic nephropathy and the adoption of the indicators in this study as diagnostic criteria for diabetic nephropathy It is possible to count the biomarkers of the current study as diagnostic signs of diabetic nephropathy in addition to other biochemical tests, and there is a strong relationship between interleukins, especially interleukin-18, and structural and functional deterioration or damage to the kidneys of diabetic patients.

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## AUTHOR CONTRIBUTION

Ali M. Hassan, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Faris S. Kata.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL CLEARANCE

The study was approved by the Ethical Approval Committee.

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