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# BIOLOGICAL SCIENCES

## PHYSIOLOGY & MOLECULARBIOLOGY

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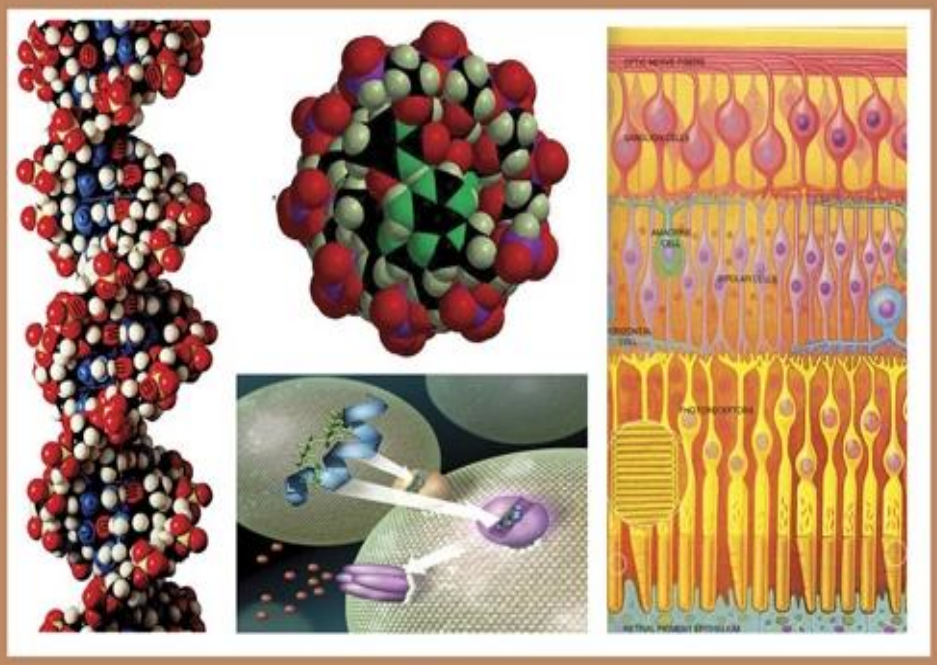

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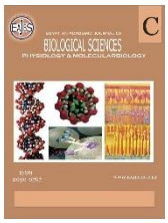

# Relation between Level of Transforming Growth Beta-1 and Micro-vascular Diabetic complications

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**ABSTRACT**

***Background:*** Diabetes mellitus (DM) is a collection of metabolic illnesses marked by chronic hyperglycemia caused by insulin production, insulin action, or both. Diabetes is classified into three types: type 1 diabetes (T1DM), type 2 diabetes (T2DM), and gestational diabetes (GDM). Diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy are examples of microvascular complications of diabetes. TGF-1 (transforming growth factor-beta 1) is one of the most important cytokines involved in the regulation of extracellular matrix (ECM) synthesis and degradation. ***Aim of this study*:** Is to illustrate the relation between the level of serum TGF-β1 in patients with type II diabetes with and without micro-vascular complications. ***Subjects and methods:*** All 90 enrolled study subjects were divided into 3 groups. Group 1: (n=30), healthy controls, group 2: (n=30) T2DM patients without microvascular complications, and group 3: (n=30) T2DM patients with microvascular complications. Those patients were collected from Fayoum University Hospital. All patients and controls are subjected to routine laboratory tests including (performed on Beckman coulter, AU-480); fasting and postprandial blood glucose, serum creatinine, glycosylated hemoglobin, and lipid profile. Serum TGF- β1 level was measured by an ELISA kit.***Results:*** There is a statistically significant difference (*P* -value <0.03 and < 0.001) between all studied groups as regards mean FBS level with highest mean among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control. Also, there is a statistically significant higher mean of 2Hour pp level (*P* -value <0.006 and < 0.001) among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control. In addition, Also there is a very highly statistically significant difference with a higher mean of HbA1c% level (*P*-value <0.001) among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control. There is a statistically significant positive correlation (*P*-value <0.05) between TGF-β1 and each of HDL, age, and creatinine levels which indicated an increase in LDL, and creatinine levels will be associated with an increase in TGF- β1 among all studied groups. There is a statistically significant difference (*P*-value <0.001) between all studied groups as regards mean creatinine level with highest mean among diabetic with microvascular complications group followed by diabetic and lower mean among control. ***Conclusion:*** This study indicated that the serum TGF-β1 level in T2DM patients with microvascular complications was significantly increased compared to T2DM patients without microvascular complications.

## INTRODUCTION

Diabetes mellitus (DM) is a diverse metabolic disease characterized by chronic hyperglycemia caused by deficiencies in insulin secretion, insulin action, or both (Marso, G.H., *et aL.*, 2016). Diabetes-related complications are associated with significant morbidity and mortality, as well as decreased life expectancy and quality of life. Diabetes affects 415 million adults today, with a projected increase to 642 million by 2040 (Ogurtsova K., *et al.*, 2017).). Diabetes is classified into the following broad categories; 1-Type 1 diabetes (owing to autoimmune death of beta cells, usually resulting in total insulin deficiency). 2-Diabetic type 2 (due to a progressive loss of b-cell insulin secretion frequently on the background of insulin resistance). 3-Diabetes Mellitus during pregnancy (GDM) (diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation. 4-Diabetes caused by other factors, such as monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation) (American Diabetes Association. (ADA). (2019). Diabetes-related complications include damage to the eyes, kidneys, and nerves as a result of small blood vessel damage. Damage to the blood vessels in the retina of the eye causes diabetic retinopathy, which can lead to vision loss and finally blindness. Diabetic nephropathy, or kidney injury, can cause scarring of the tissues, Urinary protein loss, and chronic renal disease, necessitating dialysis or transplantation of the kidneys. The most widely used complication of diabetes is nerve damage, also known as diabetic neuropathy. Numbness, tingling, pain, and altered pain sensation are some of the symptoms, which can cause skin damage (Doggen K, *et al.*, 2013).).

Transforming Growth Factor – Beta (TGF-β) is a multifunctional cytokine that regulates a variety of biological processes including immunity, differentiation, tumor suppression, tumor metastasis, senescence, migration, wound healing, apoptosis, cell division, adipogenesis, and osteogenesis. Furthermore, TGF-1 mRNA and protein are increased in the kidneys of diabetic patients, and it promotes the synthesis and cross-linking of extracellular matrix (ECM) (Wang YW., *et al.*, 2016).

## MATERIALS AND METHODS

Between September 2017 and February 2018, researchers at Fayoum University Hospital in El Fayoum, Egypt, conducted this research. The participants in this study were divided into three groups: Group 1: (n=30) healthy controls, 15 (50 percent) females and 15 (50 percent) males over the age of 30, group 2: (n=30) T2DM patients without microvascular complications, 18 (60 percent) females and 12 (40 percent) males over the age of 30, and group 3: (n=30) T2DM patients with microvascular complications, 11 (36.7%) females and 19 (63.3%) males over the age of 30. The study was approved by the Faculty of Medicine's Ethical Committee and carried out in accordance with the Helsinki Declaration (2009). In every case, their legal guardians gave their informed verbal consent. Patients who were not included in the study were: 1-patients under the age of 30. 2-patients who have other illnesses. 3-Diabetic patients with type 1 diabetes.

**Blood Samples Collection and Storage:**

Blood samples were withdrawn from each subject, part of the blood was taken on EDTA for evaluation of 1-HbA1c, part of blood sample was separated for evaluation the following parameters; 2-Glucose (directly estimated

after separation of serum sample (FBS), then another sample was withdrawn after 2hours from each subject for estimation of (2hpp). 3-serum creatinine. 4-lipid profile (cholesterol, triglycerides, HDL, and LDL). after those sera were stored at -70 Ċ until the time of analysis. this serum was used in the estimation of 5-TGF-Beta1 by ELISA Technique.

**Statistical Analysis:**

Data were collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using Statistical Package of Social Science (SPSS) software version 18 in windows 7. Quantitative data included in the study was first tested for normality by the One-Sample Kolmogorov-Smirnov test in each study group then inferential statistic tests were selected. Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement, standard deviations as a measure of dispersion for quantitative parametric data.

**For Quantitative Parametric Data:**

In-dependent **student *t*-Test** was used to compare measures of two independent groups of quantitative data

**For Qualitative Data:**

- **Chi-square** test to compare two qualitative groups.

Bivariate Pearson correlation test to test the association between variables.

- The **P-value< 0.05** was considered the cut-off value for significance.

## RESULTS

**Variations in Glucose Levels Among the Studied Groups:**

The statistical analysis indicated that there was a significant increase (*P*< 0.001) in the levels of FBS, 2hpp and HbA1c when comparing either group 2 or 3 with group 1, the control group. Additionally, the levels of FBS (*P*< 0.03), 2hpp (*P*< 0.006) and HbA1c (*P*< 0.001) were significantly higher in group II (DM) compared to group III that had micro-vascular diabetic complications (Table 1 and Figs.1-3). As mentioned above there is a statistically significant difference between all studied groups as regards mean FBS level with the highest level among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control. In addition, there are statistically significant higher levels of 2hPP (*P*-value <0.006 and < 0.001) among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control. In addition, there is a very highly statistically significant difference with higher values ofHbA1c% (p-value <0.001) among diabetic with microvascular diabetic complications group followed by diabetic and lower mean among control.

**Table 1:** Mean ± SD serum glucose level in different studied groups.

| Groups / Variables | **Group I** Control (N=30) | **Group II** DM (N=30) | **Group III** Micro-vascular diabetic complications (N=30) | **p-value** | **Sig.** |
|---|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | | |
| **FBS** (mg/dl) | 94.4±9.6 | 191.3±45.2 | **223.5±68.9** | **0.037[a] <0.001[b, c]** | **vHS** |
| **2hpp**(mg/dl) | 119.3±7.7 | 260.1±19.6 | **301.9±84.9** | **0.01 [a] <0.001[b, c]** | **HS vHS** |
| **HbA1C%** | 4.4±0.5 | 7.4±0.5 | **8.5±1.7** | **<0.001[a, b,c]** | **vHS** |

P[a]: DM compared to DM with microvascular complications

P[b]: DM compared to the control group

P[c]: DM with microvascular complications compared to controls

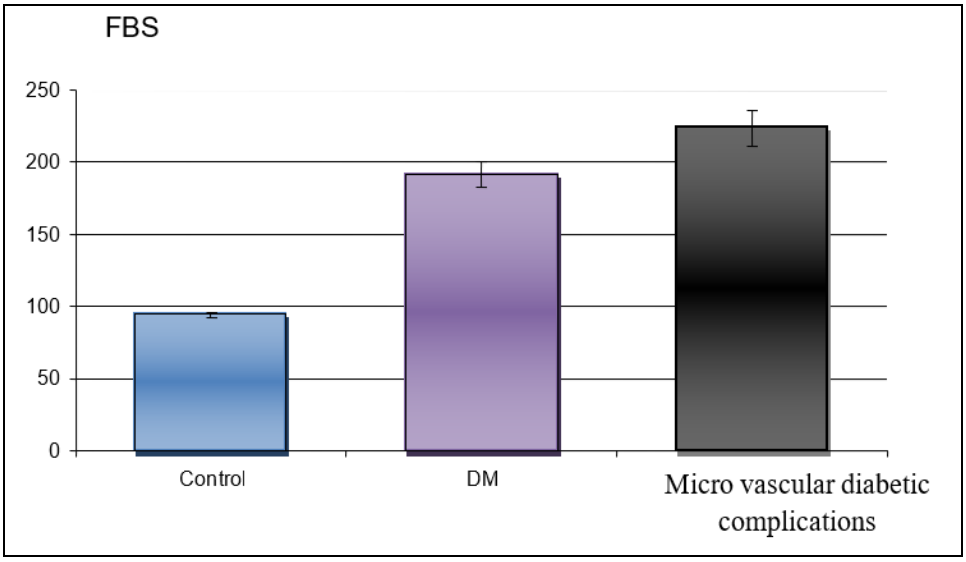


**Fig. 1:** Mean ± SD FBS in the studied groups

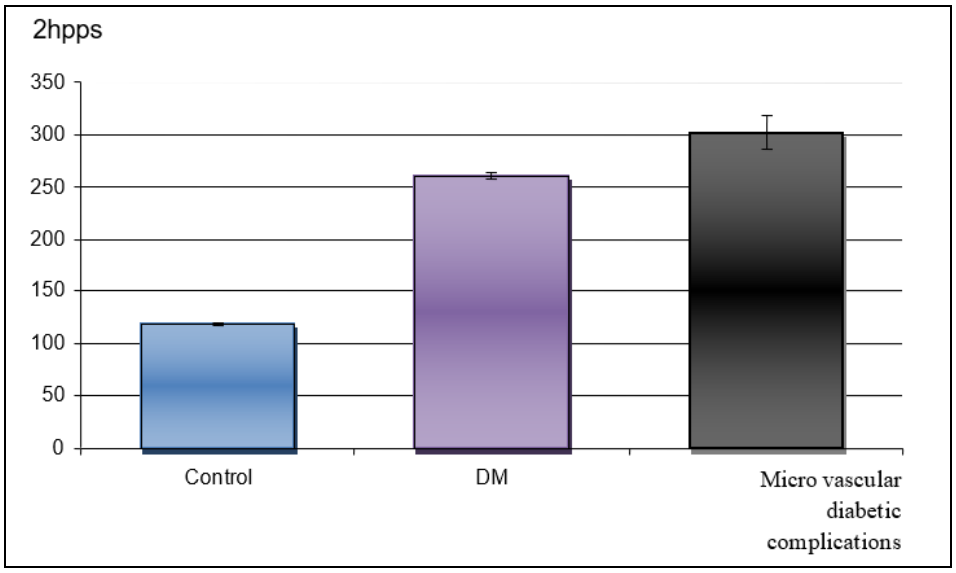


**Fig. 2:** Mean ± SD 2hpps in the studied groups.

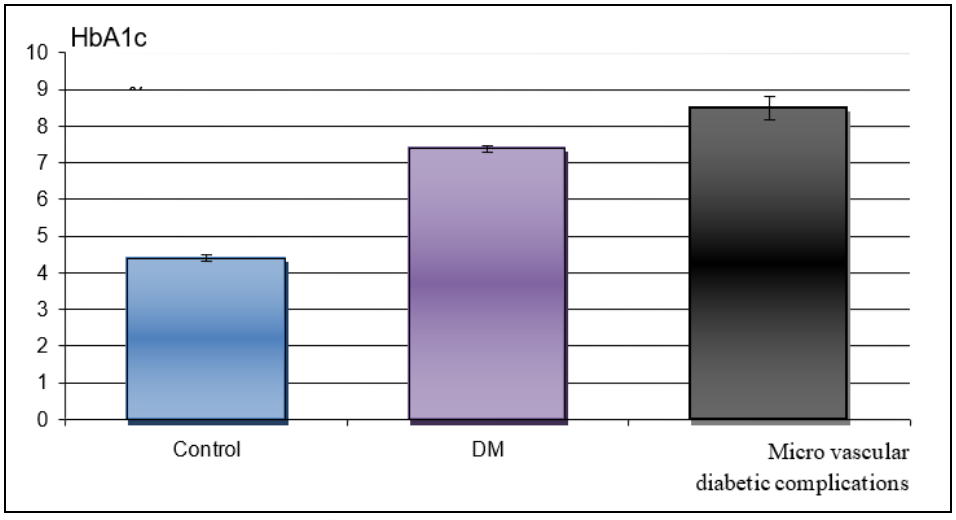


**Fig. 3:** Mean ± SD HBA1c% in the studied groups

**Serum Creatinine Levels in The Studied Groups:**

The statistical analysis showed that there was a very high significant increase ($P$ < 0.001) in serum creatinine levels in group 3 of DM with microvascular complications compared to group 1 (the control) and group 2 of DM (Table 2 and Fig.4). The difference between groups 1 and 2 is not significant ($P$ = 0.1).

**Table 2:** Mean ± SD serum creatinine levels in the studied groups.

| Variables / Groups | Creatinine level (mg/dl) | | p-value | Sig. |
|---|---|---|---|---|
| | Mean | ± SD | | |
| Control **Group (I)** | 1.5 | ±1.3 | **<0.001** [a] 0.1[b] **<0.001**[c] | **VHS** NS **VHS** |
| DM **(Group II)** | 0.75 | ±0.1 | | |
| Micro-vascular diabetic complications **(Group III)** | **5.5** | ±1.9 | | |

P[a]: DM compared to DM with microvascular complications
P[b]: DM compared to the control group
P[c]: DM with microvascular complications compared to controls
DM=Diabetes Mellitus, SD=Standard Deviation

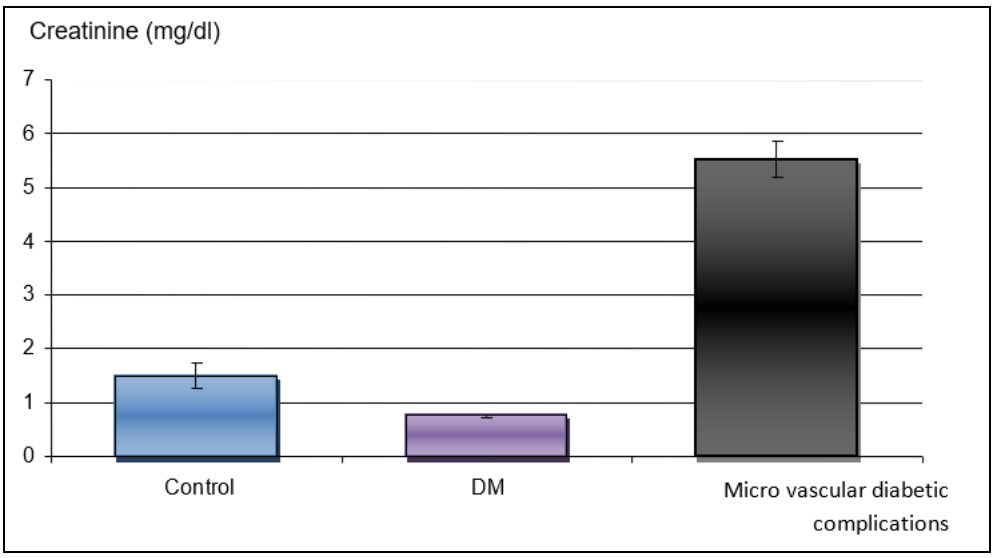


**Fig. 4:** Mean ± SD serum Creatinine levels in the studied groups**.**

**Variations in TGF-β1 Levels Among the Studied Groups:**

The highest TGFβ1 levels were recorded from group **III** of diabetic patients with microvascular complications. The statistical analysis showed that there was a highly significant increase ($P$ < 0.001) in TGF-β1 levels in group 3 of DM with microvascular complications compared to group 1 (the control) and group 2 of DM (Table 3 and Fig.5). The difference in TGF-β1 levels between groups 1 and 2 is not significant ($P$ = 0.9).

**Table 3:** Mean ± SD TGFβ1 level in the studied groups.

| Variables / Groups | TGFβ1 level (ng/ml) | | p-value | Sig. |
|---|---|---|---|---|
| | Mean | ± SD | | |
| Control **Group (I)** | 22899.1 | ±14810.1 | | |
| DM **(Group II)** | 22327.1 | ±7902.7 | **<0.001** [a] | **VHS** |
| Micro-vascular diabetic complications **(Group III)** | 53836.9 | ±16218.8 | 0.9[b] NS<br>**<0.001**[c] | **VHS** |

P[a]: DM compared to DM with microvascular complications
P[b]: DM compared to the control group
P[c]: DM with microvascular complications compared to controls
DM=Diabetes Mellitus, SD=Standard Deviation.

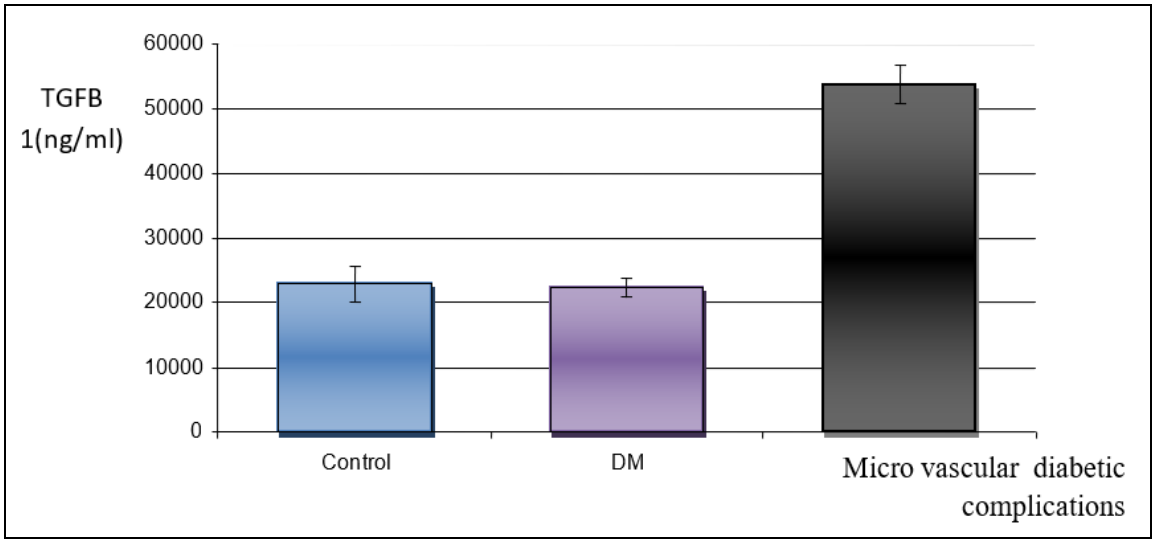


**Fig. 5:** Mean ± SD TGFβ-1 level in the studied groups.

**Correlation between TGF-β1 levels with age, anthropometric measures and routine laboratory investigations among all studied groups.**

Among the control group (I) there is a statistically significant positive correlation, between TGFβ1 and each of cholesterol (*P* =0.001), HDL (*P* =0.0, Table 4) and LDL level (*P* =0. 002;).It indicated that the increase in cholesterol, HDL and LDL was associated with an increase in TGFβ1. Also, among the diabetic group (II), there is a statistically significant positive correlation between TGFβ1 and each of BMI (*P* =0.03), cholesterol levels (*P* =0.004), and LDL levels (*P* =0.01) level. This analysis indicated that the increase in cholesterol, and LDL and BMI were associated with an increase in TGFβ1 (Table 4). On the other hand, there are no statistically significant correlations (*P*-value >0.05) between TGFβ1and other variables among diabetic with microvascular complications group III (Table 4).

**Table 4:** Correlation between TGFβ1 level with anthropometric measures and routine laboratory investigations of each of the studied groups.

| Groups / Variables | TGFβ1 level (ng/ml) | | |
|---|---|---|---|
| | **Group I** Control | **Group II** DM | **Group III** Micro-vascular diabetic complications |
| | **r(p-value)** | **r(p-value)** | **r(p-value)** |
| BMI (kg/m$^2$) | 0.17(0.4) | **-0.40(0.03*)** | -0.20(0.3) |
| **Lipid profile** | | | |
| Cholesterol (mg/dl) | **0.57(0.001*)** | **0.52(0.004*)** | -0.13(0.5) |
| TG (mg/dl) | 0.16(0.4) | 0.30(0.1) | -0.18(0.3) |
| HDL (mg/dl) | **0.45(0.01*)** | 0.01(0.9) | 0.14(0.5) |
| LDL (mg/dl) | **0.55(0.002*)** | **0.46(0.01*)** | -0.04(0.8) |
| **Glucose profile** | | | |
| FBS (mg/dl) | -0.002(0.9) | -0.22(0.3) | 0.11(0.5) |
| 2hpp(mg/dl) | -0.03(0.9) | -0.24(0.2) | -0.14(0.5) |
| HbA1C% | 0.17(0.4) | -0.19(0.3) | 0.05(0.8) |
| **Other** | | | |
| Creatinine level(mg/dl) | 0.02(0.9) | -0.06(0.8) | 0.08(0.7) |

## DISCUSSION

The current study aims to demonstrate the link between TGF-1 levels and microvascular diabetic complications. Diabetes mellitus (DM) is a diverse metabolic disease characterized by chronic hyperglycemia caused by defects in insulin secretion, insulin action, or both (Marso, G.H. *et aL.,* 2016).). Diabetes-related problems are associated with significant morbidity and mortality, as well as decreased life expectancy and quality of life. According to estimates, 415 million adults have diabetes, with this figure expected to rise to 642 million by 2040 (Ogurtsova K., *et al.*, 2017).). Diabetes has both acute and chronic complications. Diabetes patients may experience acute fluctuations in blood glucose levels that necessitate medical attention. Acute complications are more likely in patients with type 1 diabetes because they require insulin administration. If left untreated, they can be fatal. Diabetes causes sensory and motor function loss, as well as poor circulation in the hands and feet, raising the risk of infection, poor wound healing, and eventual amputation. Diabetes is a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), both of which necessitate dialysis or transplantation. Diabetes-related retinopathy, cataracts, and glaucoma are all common, causing visual disturbance and blindness. Diabetes increases the risk of coronary heart disease, heart failure, and stroke by two to four times compared to non-diabetics. There is also mounting evidence of a link between diabetes and cancer, owing to the fact that diabetics live longer lives and are less likely to die from cardiovascular disease (CVD) or chronic kidney disease (CKD) (Gregg, E. W., *et al.*, 2016).). Furthermore, diabetes and cancer share risk factors such as advanced age, tobacco use, and oxidant-rich, low-fiber diets (Giovannucci, E., *et al.*, 2010). TGFbeta-1 is a multifunctional cytokine that regulates immune differentiation, tumor suppression, tumor metastasis, senescence, migration, wound healing, apoptosis, cell division, adipogenesis, and

osteogenesis, among other biological processes (Grafe I.,*et al.*, 2014).). The subjects for this study were recruited from Fayoum University Hospital between September 2017 and February 2018. According to the findings of this study, diabetic patients with microvascular complications have a higher BMI than nondiabetic patients. The current finding is consistent with the findings of (Hu EA., *et al.*, 2012). ), who investigated the effect of BMI at the time of DM diagnosis and its complications in men and women, and discovered that excess weight and obesity are major contributors to type 2 DM and its complications in both men and women. Both men and women in the overweight category (25 BMI 29.99) had a 30% and 10% higher risk of developing diabetes, respectively. At 30 BMI 39.99, both genders were 100 percent more likely to develop diabetes than counterparts with a normal BMI. BMI 40 increases the risk of developing diabetes by up to 150 percent in women and 180 percent in men. Maintaining a healthy weight, among other things, maybe more important to women than to men at lower levels of excess weight, because DM complications are associated with even slightly overweight status (25 BMI 27.49). However, for men, the increased risk of DM complications does not occur until BMI 27.5. Obese people (BMI 30) were consistently at a higher risk of DM complications—up to 168 percent higher for all complication types combined, regardless of gender. Furthermore, obese men have a higher risk of developing cardiovascular, renal, ocular, and lower extremity complications than women with the same BMI (Gray, N., *et al.*, 2015).). High levels of TG, cholesterol, LDL, and low levels of HDL were found to be associated with microvascular diabetic complications in the current study. The findings of this study agree with those of Yang *et al.*, 2019, who found that higher levels of TG, cholesterol, LDL-C, and lower levels of HDL-C were associated with DKD. Notably, such associations were unaffected by a variety of confounding factors, including age, gender, smoking status, diabetes family history, duration of diabetes, current medical treatment, hypertension, stroke, BMI, and HbA1c level. Serum TGF-1 levels were measured using an ELISA method in T2DM (group II), T2DM with microvascular complications (group III), and healthy control (group I) subjects in the current study. The current study found a statistically significant difference (P value=0.001) in the serum levels of TGF- between study groups, III and I. TGF-1 levels in the blood were found to be significantly higher in the diabetic microvascular group (group III). These findings are consistent with those of Zhou., Tianbiao *et al.*, 2018. who investigated the relationship between serum TGF-1 levels in patients with diabetes or diabetic microvascular complications? They discovered that serum TGF-1 levels were significantly higher in T2DM and T2DM with microvascular complications. They also discovered that TGF-1 can stimulate the transcription of ECM proteins and that higher levels of TGF-1 are linked to ECM accumulation, fibrosis, and glomerulosclerosis. These findings are also consistent with those of Qiao *et al.*, 2017 and Mou *et al.*, 2016, who investigated the relationship between serum TGF-1 levels and the risk of diabetic microvascular complications. According to their findings, increased serum TGF-1 levels in diabetic patients were associated with an increased risk of diabetic microvascular complications involvement. The findings of this study contradict those of Castro *et al.*, 2014, who found an increase in TGF-1 in T2DM patients with microvascular complications when compared to T2DM patients and healthy patients, but the differences were not statistically significant. This finding was supported by a previous study by Kim and Frankel (2011).

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