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Levels of Growth Differentiation Factor 15 and Interleukin-29 in Children and Adolescents with Type 1 Diabetes Mellitus

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ABSTRACT

Background: Micro-vascular complications of type 1 diabetes are characterized by damage to the microvasculature of the kidney, retina, and neurons. Some proteins are interesting in their biological functions and can be engaged in the development of micro-angiopathy such as growth/differentiation factor 15 (GDF-15) and interleukin 29 (IL-29).

Aim: This study aimed to investigate the diagnostic value of growth/differentiation factor 15 (GDF-15) and interleukin 29 in cases of type one diabetes and its relation to micro-vascular complications.

Subjects and Methods: This study included 80 participants that were divided into 3 groups. Group 1 included 20 patients with T1DM who had micro-vascular complications, group 2 contained 20 patients with T1DM who did not have microvascular complications and group 3: 40 healthy controls. All participants were subjected to full history taking and thorough clinical examination. Laboratory investigations included HbA1c, fasting lipid profile, micro-albumin in random urine sample, eGFR calculation, GDF-15 and IL-29 assay using ELISA kit.

Results: There was a statistically significant difference between patients and control group as regards GDF-15 and IL29 (p= 0.041 and 0.013, respectively) which were higher in patients group. A high statistically significant difference was found between both groups as regards levels of GDF-15, which was higher in diabetic patients with complications than patients free of complications (p= 0.001). But no statistically significant difference between both patients' groups as regards levels of IL-29 (P > 0.05).

Conclusion: Our results strongly indicate that in T1DM, serum GDF-15 level might serve as a useful marker to detect micro-angiopathy such as diabetic kidney disease, peripheral neuropathy, and retinopathy.

Keywords: T1DM, GDF-15, Interleukin-29, microvascular complications, diabetes.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic disease characterized by increased blood glucose levels due to insulin deficiency that occurs as a consequence of loss of the pancreatic islet beta cells (β-cells)**.** It is one of the most common and serious long-term diseases present in childhood. Hyperglycemia is the primary risk factor for microvascular as well as macrovascular disease, and decreasing HbA1c and keeping the blood glucose level within the target range (TIR) through intensive diabetes management, particularly at the onset of the disease, is associated with striking (approx. 70%) reductions in incidence and slower progression of microvascular disease. However, differences in HbA1c do not fully explain the variation in the incidence of complications and the severity of disease between individuals **(1).**

Cardiovascular disease remains the major cause of premature morbidity and mortality, suggesting an average of 10 years shorter life expectancy for people with T1DM than for healthy individuals **(2).** Microvascular disease tends to occur mainly in tissues where glucose uptake is independent of insulin activity as retina, kidney and vascular endothelium because these tissues are exposed to glucose level that correlates very closely with blood glucose level **(3).**

Hyperglycemia promotes microvascular tissue damage through five main mechanisms; an increased flux of glucose through the polyol pathway, an increased formation of intracellular advanced glycation end products (AGEs), interaction between AGEs and their receptors leading to intracellular signaling that disrupts cell function, a persistent activation of protein kinase C (PKC), and an increased activity of hexosamine pathway. These metabolic injuries lead to altered blood flow and changes in endothelial permeability, extravascular protein deposition and coagulation leading to organ dysfunction **(4).** The major microvascular complications of T1DM are nephropathy, retinopathy and neuropathy **(5)**.

Despite very important progress in the control of the disease and novel therapeutic options, the occurrence of microvascular complications still cannot be fully prevented **(6)**. So, we need new biomarkers for early diagnosis of microvascular complications and guide the application of new therapeutic options for preventing development and progression of complications **(7, 8).**

Some proteins are interesting in their biological functions and can be engaged in the development of micro-angiopathy such as growth/differentiation factor 15 (GDF-15) and interleukin 29 (IL-29) **(9).**

GDF15 mRNA is translated into a 308-amino-acid protein, containing a 167-amino-acid pro-peptide that dimerizes and is processed at a conserved pro-convertase site to be secreted as a readily diffusible,

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approx. 25-KDa dimeric cysteine knot protein of 224 amino acids with structural characteristics typical of the TGF-β superfamily [10].

GDF-15 levels are increased in various pathological conditions and diseases, including inflammation, cardiovascular disease, renal disease, pulmonary disease, and cancer [11]. GDF-15 is produced in activated macrophages, and in pathological conditions including pro-inflammatory status, vascular injury, pressure overload, and oxidative stress from human endothelial cells, vascular smooth muscle cells, and adipocytes. GDF-15 is expressed in most human tissues in response to various signals, including tissue damage, hypoxia, mechanical compression and many more. The protein biosynthesis of GDF-15 is well-documented and includes pro-GDF15 dimer and mature GDF-15 synthesis. It is still not very clear which of these protein forms are functionally relevant as they both are secreted in the plasma. Once released, they have a plethora of effectors, which can be broadly classified as defined and undefined mechanisms. GDF-15 has been shown to interact with 3 distinct receptors i.e. GFRAL, ErbB2 and CD44, which have been reported to have different effects. The expression of GDF-15 in various tissues suggests its importance in general and basic cellular functions. Although the exact biological functions of GDF-15 remain largely unclear, it has been demonstrated to modulate inflammatory, apoptotic, and angiogenesis pathways [12].

Interleukin-29 (IL-29, also known as interferon lambda 1, IFN-λ1) is a new member of the recently discovered interferon lambda (IFN-λ) family. IL-29 is closely related to the interleukins IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A and IL-28B. All mentioned proteins belong to a large family called the IL-10 family. IFN-λ proteins are part of the class II cytokines. All these cytokines act through the class II cytokine receptor family (CRF2). Hence, IFN-λ and the IL-10 family are sometimes referred to as CRF2 cytokines [9].

Dendritic cells and macrophages produce it as a response to autoimmune processes or viral infections [13]. Elevated Il-29 levels were detected in the following autoimmune disorders: Sjögren syndrome, rheumatoid arthritis, systemic sclerosis, and psoriasis. Increased concentrations of this interleukin were also found in atopic dermatitis and asthma [14].

In this study, we investigated the diagnostic value of growth/differentiation factor 15 (GDF-15) and interleukin 29 in the diagnosis of early vascular complications in young patients with T1DM. The present study aims to investigate whether these inflammatory proteins growth/differentiation factor 15 (GDF-15) and interleukin 29 are related to the prevalence of microvascular complications and markers of glycemic control in T1D patients.

Study design:

This cross-sectional study included 40 children and adolescents with type one diabetes with disease duration more than 5 years. Pediatric age group ranged from 7 to 18) years. They were recruited from the Paediatric and Adolescents Diabetes Clinic, Ain Shams University Hospital. Forty age and sex matched healthy controls were recruited from outpatient clinics in Children Ain Shams University Hospitals. All participants were divided into 3 groups [15].

Group 1: 20 diabetic patients have micro-vascular complications.

Group 2: 20 diabetic patients did not have micro-vascular complications.

Group 3: 40 are healthy controls.

Inclusion Criteria: Diagnosis of T1DM was done based on International Society for Pediatric and Adolescent Diabetes (ISPAD) criteria of diabetes diagnosis in children, 2018. Children and adolescents with type one diabetes for more than 5 years.

Exclusion Criteria:

- Acute inflammation. Diabetic ketoacidosis or ketonurea. Neoplasm. Autoimmune disorders as Sjögren syndrome, rheumatoid arthritis, systemic sclerosis, psoriasis, thyroid disorders, and celiac disease. Atopic dermatitis and asthma. Pulmonary disease.
-

All participants were subjected to:

Full history taking including: duration of diabetes. Symptoms of neuropathy as numbness, tingling, ulcers, and pain. Symptoms of nephropathy as hypertension, presence of lower limb edema. Symptoms of retinopathy as blurring of vision and headache. Absence of primary causes of micro-vascular complication rather than diabetes.

Physical Examination

Each participant underwent full physical examination including chest, abdominal and neurological examination, blood pressure assessment and anthropometric measurements **(**Weight, height and body mass index (BMI) percentile plotting according to appropriate age and sex). Muscles strength and tone, tendon reflexes, sensitivity to touch and vibration, presence of lower limb edema and eye examination. Ophthalmological examination was performed using direct ophthalmoscopy by a single person specialized in ophthalmology according to the American Academy of Ophthalmology guidelines. The diagnosis of diabetic peripheral neuropathy based on American Diabetes Association diagnostic criteria; two or more out of the following criteria are required to diagnose: symptoms reported by the patient, abnormal touch sensation, abnormal feeling of

vibration, abnormal temperature sensation, and abnormal Achilles tendon reflexes.

Laboratory Investigations

Under complete aseptic conditions, 10 mL of fasting venous blood were obtained by a clean venipuncture, two milliliters were placed in EDTA tube for subsequent assay of HbA1c, while the rest was evacuated in two plain test tubes. The serum was separated by centrifugation (1000x g for 15 minutes). Serum of one tube was immediately assayed for lipid profile, while the serum collected in the other tube was divided in three aliquots and stored at -20ºC for subsequent assay of fasting C-peptide, serum lipase and trypsinogen. Hemolysed samples were discarded. Repeated freezing and thawing was avoided.

A. HbA1c was assayed by high performance liquid chromatography (HPLC) technique on the Bio-Rad d-10 hemoglobin testing system (Bio-Rad Laboratories, Inc., 4000 Alfred Nobel Drive, Hercules, California 94547, USA). Serum samples were assayed for **lipid profile** on the Beckman AU-680 system auto-analyzer (Beckman Coulter, Inc. Diagnostics Division Headquarters 250 South Kraemer Boulevard Brea, California 92821-6232 USA) using reagents supplied by the company.
B. Triglycerides (TG) and total cholesterol were measured on AU680 that is based on enzymatic colorimetric method. High density lipoprotein cholesterol (HDL-cholesterol) assay is based on precipitation of low density lipoprotein cholesterol (LDL-cholesterol) and very low density lipoprotein cholesterol (VLDL-cholesterol) and then the cholesterol in the HDL cholesterol fraction which remains in the supernatant is assayed by a timed endpoint method. LDL cholesterol was calculated according to "Friedwald equation": LDL-cholesterol = Total cholesterol-(HDL-C+TG/5), provided that serum TG is ≤ 400 mg/dL, samples with TG>400 mg/dL are diluted according to its concentration.
C. Albumin excretion rate (AER): Using immune turbidimetric methods. It is used to assess presence of nephropathy. Patients initially diagnosed as having microalbuminuria with AER >30 mg/mg creatinine were asked to perform two further urine collections at intervals of 3 - 6 months. Persistent microalbuminuria was defined when two of three samples showed an albumin excretion rate of 30 - 300 mg/mg creatinine.
D. Blood samples were obtained and analyzed for concentration of the GDF-15 and IL-29 (ELISA Kit).

Ethical considerations:

The study was approved by Ethics Committee of Faculty of Medicine, Ain Shams University. Informed consents were obtained from the guardian of each participant included in the study. The Helsinki Declaration, the World Medical Association's code of ethics for human studies, directed the conduct of this investigation.

Statistical Analysis

The collected data was revised for completeness and accuracy. Data were coded, entered and analyzed using the statistical software (SPSS 23) for data analysis. Quantitative data were expressed as mean and standard deviation (SD) and qualitative data were expressed as number (n) and percentage (%). Student's t-test was applied in case of statistical comparison between quantitative parametric data of two independent groups. Chi-square test was used to compare categorical and qualitative data. Mann–Whitney U test was used to compare quantitative nonparametric data from two independent groups. $P \leq 0.05$ was considered significant.

RESULTS

Our study involved forty young patients with T1DM, 22 females and 18 males, with mean age of 14.05 ± 2.42 years old and with mean disease duration 8.05 ± 2.10 years. They were compared to 40 age- and sex-matched healthy controls. There was a statistically significant difference between patients and control group as regards GDF-15 and IL-29 (p= 0.041 and 0.013, respectively) which were higher in patients group as shown in table (6) and figures (1 & 2).

Table (1) showed that there was no statistically significant difference between controls and patients group regarding sex and mean age but there was statistically significant difference between patients and controls regarding BMI which was increased in the patients' group (p = 0.010) .

The patients' group was further divided into 2 groups, according to glycemic control, into controlled with HbA1c < 7% and uncontrolled group with HbA1c > 7% as shown in tables (2, 3 & 4).

A high statistically significant difference was found between both groups as regards presence of microalbuminuria, TDD and DM duration (p= 0.001, 0.010, 0.032, respectively), which was increased in uncontrolled group (HbA1C >7%). Meanwhile, no statistically significant difference was detected regarding presence of retinopathy, neuropathy, and hypertension. There was a statistically significant difference between both groups as regards levels of cholesterol, LDL-C and eGFR (p= 0.020, 0.001, 0.028, respectively) in which LDL-C and cholesterol being higher and eGFR being lower in the uncontrolled group (HbA1C>7%).

In addition, no statistically significant difference was detected regarding triglycerides, HDL-C, TSH and CRP. A highly statistically significant difference was observed between patients' subgroups regarding GDF-15 (p= 0.004), which was higher in uncontrolled group (HbA1C>7%) as shown in figure (2). However, no

statistically significant difference was observed between both subgroups as regards IL-29.

Furthermore, our studied patients were divided into 2 groups according to the presence or absence of documented microvascular complications (diabetic retinopathy, diabetic nephropathy or diabetic peripheral polyneuropathy) as shown in tables (4 and 5). Clinical and laboratory data showed a highly statistically significant difference between patients' groups regarding presence of HTN, DM duration, cholesterol, HbA1c, eGFR and TDD.

HTN (P= 0.041) which presented more in diabetic patients with complication (27.5% of the patients) than those free of complications (4.5%).

Diabetes duration was longer in diabetic patients with complications than diabetic patients without complications (P= 0.001). Levels of cholesterol, HbA1c, eGFR and TDD (P= 0.001, 0.001, 0.017, 0.010, respectively) were higher in diabetic patients with complications. Meanwhile, no statistically significant difference was detected regarding HDL-C, LDL-C, triglyceride, TSH and CRP. A high statistically significant difference was found between both groups as regards levels of GDF-15 which was higher in diabetic patients with complications than in patients free of complications (p= 0.001).

But no statistically significant difference between both patients' groups as regards levels of IL-29 (P > 0.05). On studying the correlation between levels of GDF-15 and other studied parameters, we found a positive correlation between GDF-15 concentration and age, DM duration, HbA1c, cholesterol and TDD (r= 0.361, 0.593, 0.003, 0.367, 0.416) and (p= 0.022, 0.00, 0.022, 0.00, 0.020, 0.008, respectively). However, there was negative correlation between GDF-15 concentration and e GFR(r=-0.720) and (p = 0.00), the later relation is illustrated in figure (3).

Table (1): Descriptive and Comparative Statistics of Demographic Data between Patients and Controls.

		Controls group	**Patients group**	**Test value**	**P-value**	**Sig.**
		No.= 40	**No.= 40**			
Sex	Female Male	21 (52.5%) 19 (47.5%)	22 (55.0%) 18 (45.0%)	0.050*	0.823	NS
Age(years)	Mean ± SD Range	13.25 ± 2.84 8 – 18	14.05 ± 2.42 8 – 18	-1.358•	0.178	NS
BMI (percentile)	Median (IQR) Range	50 (25 – 75) 3 – 95	75 (25 – 85) 10 – 95	-2.564≠	0.010	S

BMI: body mass index P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant *: Chi-square test; •: Independent t-test; ≠: Mann-Whitney test.

Table (2): Descriptive and comparative statistical analysis of clinical data between controlled and uncontrolled diabetic patients

		Controlled DM HbA1C ≤7%	**Uncontrolled DM HbA1C>7%**	**Test value**	**P-value**	**Sig.**
		No.= 11	**No.= 29**			
Presence of nephropathy	Free Microalbuminuria (30-300)mg/day	11 (100.0%) 0 (0.0%)	12 (41.4%) 17 (58.6%)	11.214*	0.001	HS
Retinopathy	Free Diabetic retinopathy	11 (100.0%) 0 (0.0%)	28 (96.6%) 1 (3.4%)	0.389*	0.533	NS
Neuropathy	Free Diabetic neuropathy	11 (100.0%) 0 (0.0%)	28 (96.6%) 1 (3.4%)	0.389*	0.533	NS
TDD (unit/kg/day)	Mean ± SD Range	0.96 ± 0.14 0.7 – 1.2	1.30 ± 0.39 0.6 – 2	-2.727•	0.010	S
DM duration(years)	Mean ± SD Range	6.91 ± 1.76 5 – 11	8.48 ± 2.08 5 – 13	-2.221•	0.032	S
Pulse	Normal	11 (100.0%)	29 (100.0%)	-	-	-
Presence of hypertension	Normal Hypertension	11 (100.0%) 0 (0.0%)	23 (79.3%) 6 (20.7%)	2.677*	0.102	NS

TDD: total daily insulin dose. P-value > 0.05: Nonsignificant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test; •: Independent t-test.

Table (3): Descriptive and Comparative Statistical Analysis of Laboratory Data between controlled and uncontrolled diabetic patients

		Controlled DM HbA1C ≤7% No.= 11	Uncontrolled DM HbA1C >7% No.= 29	Test value	P-value	Sig.
Cholesterol (< 200 mg/dL)	Mean ± SD	147.91 ± 18.10	191.97 ± 36.97	-2.422•	0.020	S
Triglyceride (< 150 mg/dl)	Mean ± SD	107.45 ± 22.86	126.07 ± 19.66	-0.839•	0.407	NS
HDL-C (>40 mg/dl)	Mean ± SD	52.27 ± 6.26	54.66 ± 13.66	-0.496•	0.623	NS
LDL-C (<130 mg/dl)	Mean ± SD	71.55 ± 8.04	92.12 ± 15.27	-3.688•	0.001	HS
eGFR (mL/min/1.73 m^2)	Mean ± SD	110.18 ± 13.04	97.76 ± 15.42	2.290•	0.028	S
CRP (mg/l)	Negative Positive	8 (72.7%) 3 (27.3%)	21 (72.4%) 8 (27.6%)	0.000*	0.984	NS

CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein;; TSH; thyroid stimulating hormone. P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant. *: Chi-square test; •: Independent t-test

Table (4): Descriptive and Comparative Statistical Analysis of GDF15 and IL29 between controlled and uncontrolled diabetic patients

		Controlled DM HbA1C ≤7 No.= 11	Uncontrolled DM HbA1C >7 No.= 29	Test value	P-value	Sig.
GDF15(ng/l)	Median (IQR) Range	250 (200 – 290) 120 – 300	300 (280 – 400) 200 – 1250	-2.877≠	0.004	HS
IL29(ng/l)	Mean ± SD Range	253.18 ± 74.71 175 – 400	265.17 ± 77.67 180 – 600	-0.440•	0.662	NS

GDF15: gross differentiation factor 15; IL29: interleukin 29 P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant •: Independent t-test; ≠: Mann-Whitney test

Table (5): Descriptive and comparative statistics of clinical and laboratory data between Patients' Subgroups regarding presence of microvascular complications

		Not complicated No. = 22	Complicated No. = 18	Test value	P-value	Sig.
Presence of hypertension	Normal Hypertension	21 (95.5%) 1 (4.5%)	13 (72.2%) 5 (27.8%)	4.191*	0.041	S
Pulse	Normal	22 (100.0%)	18 (100.0%)	-	-	-
DM duration (years)	Mean ± SD Range	6.91 ± 1.48 5 – 11	9.44 ± 1.92 6 – 13	-4.726•	<0.001	HS
HbA1C %	Mean ± SD Range	8.64 ± 2.15 6.8 – 14	9.63 ± 0.79 8.1 – 11	-7.564•	<0.001	HS
eGFR(ml/min/1.73m^2)	Mean ± SD Range	106.59 ± 15.46 80 – 129	94.56 ± 14.74 73 – 120	2.500•	0.017	S
Cholesterol (<200 mg/dL)	Mean ± SD	156.09 ± 28.08	208.89 ± 42.91	-3.449•	0.001	HS
Triglyceride (<150 mg/dl)	Mean ± SD	119.55 ± 15.77	122.67 ± 18.68	-0.839•	0.407	NS
HDL-C (>40 mg/dl)	Mean ± SD	53.05 ± 10.20	55.17 ± 8.99	-0.496•	0.623	NS
LDL-C (<130 mg/dl)	Mean ± SD	82.84 ± 12.34	90.89 ± 9.92	-0.524•	0.603	NS
TDD (unit/kg/day)	Mean ± SD	1.08 ± 0.36	1.36 ± 0.34	-2.727•	0.010	S
CRP (mg/l)	Negative Positive	14 (63.6%) 8 (36.4%)	15 (83.3%) 3 (16.7%)	1.926*	0.165	NS

BMI: body mass index; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; CRP: C-reactive protein; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TDD: total daily insulin dose; TSH; thyroid stimulating hormone; GDF15: gross differentiation factor 15; IL29: interleukin 29 P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant *: Chi-square test; •: Independent t-test

Table (6): Descriptive and comparative statistics of GDF15 and IL29 between patients' groups regarding presence of microvascular complications.

		Not complicated No. = 22	Complicated No. = 18	Test value	P-value	Sig.
GDF-15 (ng/l)	Median (IQR) Range	250 (220 – 290) 120 – 300	390 (300 – 400) 320 – 1250	-4.618≠	<0.001	HS
IL-29 (ng/l)	Mean ± SD Range	249.32 ± 62.42 175 – 400	277.22 ± 89.56 200 – 600	-0.440•	0.662	NS

GDF15: gross differentiation factor 15; IL29: interleukin 29 P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant •: Independent t-test; ≠: Mann-Whitney test.

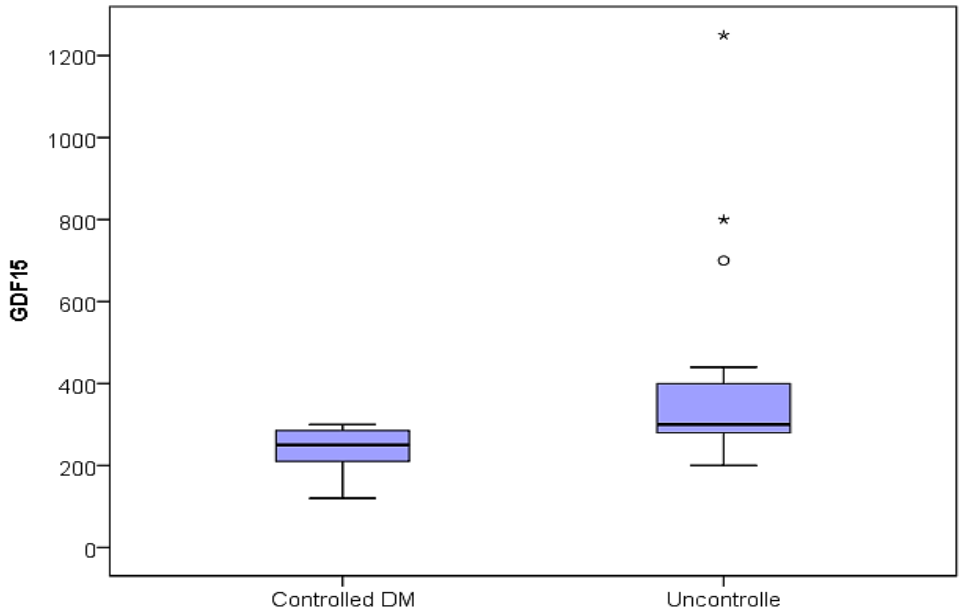

Figure (1): GDF-15 level between controlled (HbA1C≤7%) versus uncontrolled patients with T1DM (HbA1C>7%).

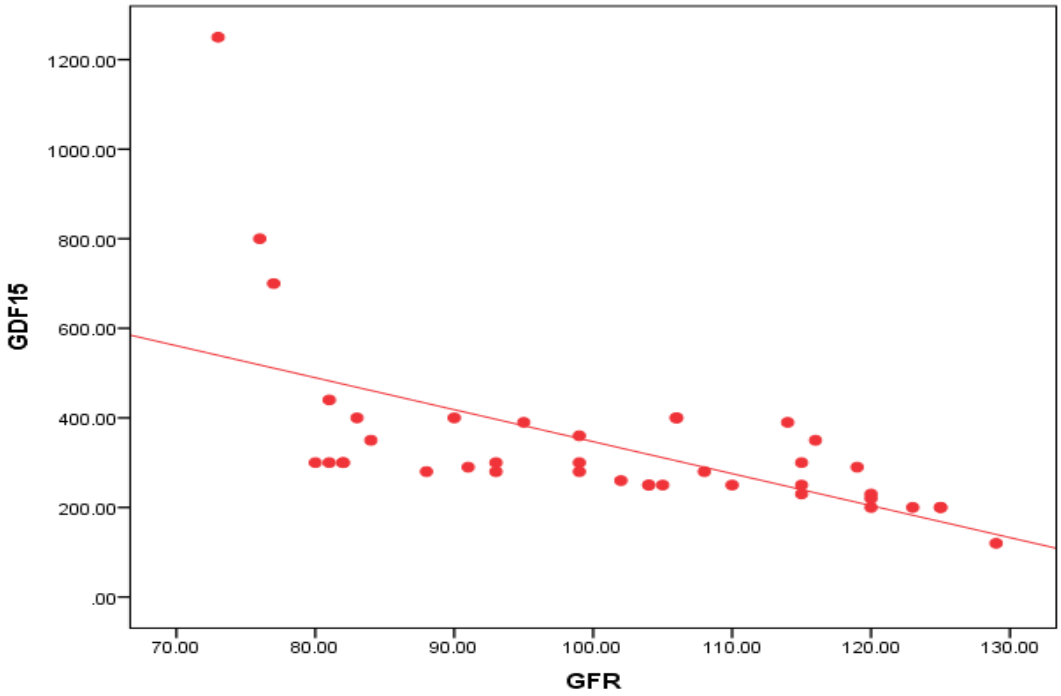

Figure (2): Comparison between control and patients' group regarding IL-29 of the studied subjects

Figure (3): Negative correlation between GDF15 and eGFR (ml/min/1.73m^2)

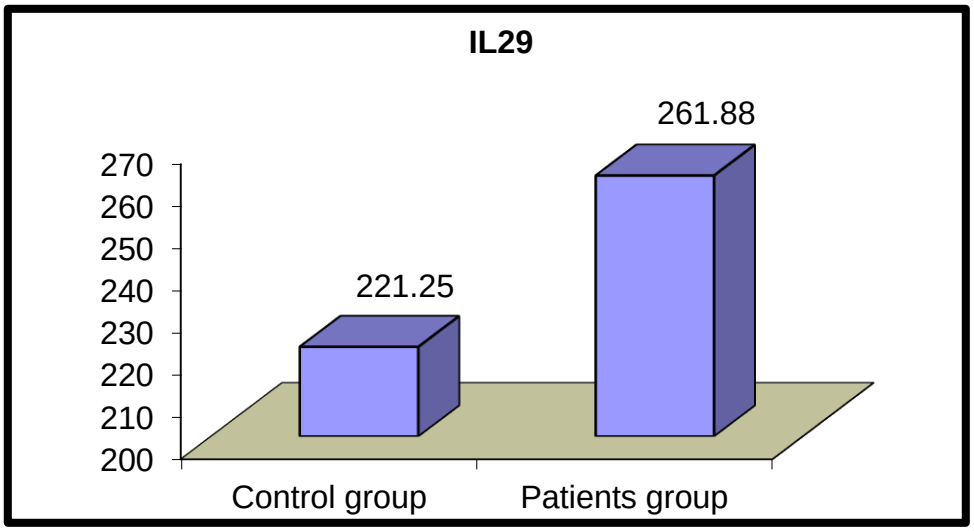

Figure (3): Negative correlation between GDF15 and eGFR (ml/min/1.73m^2)

DISCUSSION

T1DM is a chronic disease with a high risk of developing complications especially microvascular complications. Therefore, there is a rising urge for early diagnosis of T1DM and detection of its complications. The present study investigated the relation between inflammatory proteins Growth/differentiation factor 15 (GDF-15) and Interleukin 29 (IL-29) and glycemic control and prevalence of microvascular complications in T1DM patients.

This study was conducted on 40 T1DM pediatric and adolescent patients and 40 healthy, age- and sex-matched children and adolescents as a control group. Patients' group was subdivided into two subgroups. Subgroup Ia included patients without microvascular complications and subgroup Ib included patients with microvascular complications.

In the present study, the body mass index (BMI) percentile values were significantly higher in patients with T1DM than in controls. This finding is in agreement with that of **Fellinger *et al.*** [16] who observed higher BMI percentiles among adolescents and young adults with T1DM as compared to healthy subjects. The increase in weight in T1DM could be explained by the intensive use and high doses of exogenous insulin therapy in the management of T1DM which in turn has an anabolic effect on lipid metabolism thus promoting lipogenesis and weight gain. Moreover, insulin resistance may develop in T1DM especially with onset of puberty and long duration of the disease with characteristic abdominal obesity and liability for development of metabolic syndrome [17, 18].

As regards the clinical and laboratory findings, serum levels of cholesterol, triglyceride, LDL-C and CRP were significantly higher in patients group than controls. On the other hand, when the two patients' subgroups were compared to each other concerning their demographic characteristics, clinical and laboratory findings, it was found that level of cholesterol, diabetes disease duration, HbA1c and presence of hypertension were significantly higher in patients with microvascular complication than those without complications. The mean value of HbA1c in patients' with microvascular complications was 9.6% indicating poor control of diabetes. These findings are in accordance with previous study by **Ramanathan** [19] who reported that long diabetes duration; poor glycemic control and hypertension are risk factors for the development of microvascular complications. Our study observed that there was highly significant difference between patients' subgroups regarding cholesterol level which were higher in patients with microvascular complication ($p = 0.001$) this finding is in accordance with previous study by **Yang *et al.*** [20].

The mean serum GDF15 level was significantly higher in T1DM patients more than healthy controls ($p = 0.041$) this is in agreement with previous study by **kempf *et al.*** [21] and **Adela *et al.*** [22] who reported increase in level of GDF-15 in diabetic patients. This pro-inflammatory process is due to chronic hyperglycemic status and increased reactive oxygen species (ROS) formation leading to cellular injury and cell death. Increased ROS cells can cause apoptosis that increased GDF-15 to protect endothelial cells against cellular injury.

Also, a statistical significant difference was found in the serum level of GDF-15 in the patients' subgroups, which was higher in patients with microvascular complications more than patients free of these complications ($p = 0.001$). These findings are in accordance with previous study by **Falkowski *et al.*** [15] who reported the importance of GDF-15 as a marker of microvascular complications in T1DM. In our study GDF-15 was highly statistically significant in patients

with diabetic nephropathy. This is in accordance with findings of **Perez-Gomez *et al.*** [23] and **Yücel *et al.*** [24] who reported that albuminuria is a characteristic feature of diabetic nephropathy and is a condition associated with chronic inflammation, which is associated with increased GDF-15 level. In addition, there was no statistically significant difference between serum level of GDF-15 and other microvascular complications namely diabetic retinopathy and diabetic neuropathy. This may be due to small sample size of these complications in collected patients. This result is in contrast with **Chung *et al.*** [25] and **Falkowski *et al.*** [15] who reported that plasma GDF-15 concentrations were independently and positively associated with diabetic retinopathy and neuropathy in diabetic patients.

On the other hand, when the two patients' subgroups were compared to each other as regards their demographic characteristics, clinical and laboratory findings, it was found that diabetes disease duration, HbA1c and presence of hypertension were significantly higher in patients with microvascular complications than those without microvascular complications. The mean value of HbA1c in patients' with complications was 9.63% indicating poor control of diabetes. These findings are in accordance with previous studies by **Yang *et al.*** [26]**, Bjornstad *et al.*** [27]**,** and **Lind *et al.*** [28] who reported that long diabetes duration, poor glycemic control and hypertension are risk factors for the development of microvascular complications as diabetic nephropathy. Moreover, there was significantly statistically difference between patients subgroups regarding cholesterol, which was increased in complicated patients ($p = 0.001$). This is in accordance with **Savelieff *et al.*** [29] and **Yücel *et al.*** [24] who reported dyslipidemia as a risk factor and had a mechanism in microvascular complications.

The present study showed that there was positive correlation between level of GDF-15 and age. This finding is in agreement with **Jiang *et al.*** [30] and **Bao *et al.*** [31] who reported that age associated with GDF-15 expression via both physiological and pathological processes and elevated concentration of circulating GDF-15 could reflect mitochondrial dysfunction. Moreover, there was positive correlation between GDF-15 and hypertension as studies by **Lajer *et al.*** [32]**, Kou *et al.*** [33] and **Xiao *et al.*** [34] who reported increase level of GDF-15 in hypertensive patients. In addition there was positive correlation between hypercholesterolemia and GDF-15, which is in agreement with **Yücel *et al.*** [24] who reported increased level of GDF-15 with dyslipidemia, which cause atheromatous plaques, resulting in endothelial dysfunction and increased local inflammation, resulting in an increase in GDF-15. /Another protein investigated in the current study was IL-29, the mean serum level of IL29 significantly was higher in patients with T1DM more than healthy controls. However, no statistical significant difference was found in the mean serum IL-29 between diabetic subgroups. This finding is in agreement with **Falkowski *et al.*** [15] who reported that IL-29 does not seem to take part in micro-vessel damage in the course of diabetes and it is associated with an acute autoimmune process that is not present in patients with disease duration of longer than 5 years [15].

CONCLUSION

High levels of serum GDF-15 on the prevalence of microvascular complications in the course of T1DM are well associated. Our results strongly indicated that in T1DM, serum GDF-15 level might serve as a useful marker to detect microangiopathy such as diabetic kidney disease, peripheral neuropathy, and retinopathy. However, further and prospective studies on larger groups of patients should be performed in the future to assess the full clinical utility of these markers. This is the first study to demonstrate elevated IL-29 serum level in patients with T1DM. The background for the development of T1DM is considered to be multifactorial, while our results suggest that IL-29 might be engaged in one of the pathogenetic pathways. Future studies are required to evaluate the potential of the protein as a therapeutic target in T1DM.

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