Lack of glycemic control in type two diabetes mellitus patients is associated with reduced serum epidermal growth factor level and increased insulin resistance

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Abstract. The prevalence of type 2 diabetes mellitus (T2DM) is steadily increasing worldwide in an alarming fashion. Importantly, poor glycemic control is associated with development of various health sequalae's due to glucolipotoxicity, oxidative stress and increased inflammatory cytokines. The aim of the present study was to examine the effect of glycemic control on the relative abundance of inflammatory markers in patients with controlled and uncontrolled T2DM, and to test their association with the glycemic status in diabetic patients in Jordan. An observational cross-sectional study design was used. Patients with T2DM with controlled diabetes [glycated hemoglobin (HbA_{1c}) ≤7.0%, n=110] and age-, sex- and body mass index (BMI)-matched uncontrolled diabetic patients (HbA_{1c} >7.0%, n=105) were recruited. An antibody membrane array was used to examine the relative abundance of inflammatory cytokines and growth factors in the sera of the study subjects, followed by enzyme-linked immunosorbent assay (ELISA) to confirm the results. Fasting blood glucose, serum insulin, triglyceride and homeostatic model assessment for insulin resistance (HOMA-IR) score were significantly elevated in the uncontrolled T2DM group (P<0.05). Antibody membrane array showed that serum epidermal growth factor (EGF) is significantly decreased in the uncontrolled T2DM group, and this was confirmed by ELISA (158.77±111.7 vs. 95.9±82.7 pg/ml, P=0.002). The binary logistic model was used to predict the likelihood of being uncontrolled diabetic based on EGF levels. After controlling for age, sex and BMI, EGF was statistically associated with diabetes control, where lower EGF levels predicted uncontrolled diabetes. Additionally, Pearson's product-moment correlation showed a statistically significant negative correlation between EGF and HbA_{1c} (r=-0.25, P<0.0001), and a positive correlation between HOMA-IR and HbA_{1c}, (r=0.32, P<0.0001). The current data identify a novel link between serum EGF levels and the status of HbA_{1c} indicative of diabetic control.

Introduction

Type 2 diabetes mellitus (T2DM) is among the fastest growing global health emergencies at present, reaching alarming levels. Globally, >10% of adults are now living with diabetes. In 2021, the number of individuals living with diabetes was estimated to be 537 million (10.5% of the global population) worldwide, with an expected rise to 783 million (12.2% of the global population) by 2045 (1). The prevalence of T2DM in Jordan is the second highest DM prevalence worldwide, where it was 14.0% in 1990 and is expected to rise to 20.6% in 2050 (2). T2DM is a lifelong progressive chronic metabolic disease characterized by chronic hyperglycemia due to either impaired insulin action in peripheral target tissues, declined insulin secretion due to β-cell failure, or both (3). Indeed, T2DM increases the risk of developing various microvascular and macrovascular complications, resulting in a significant financial burden on the patients, their families, and the healthcare system (4,5).

The American Diabetes Association has classified T2DM based on the level of glycated hemoglobin (HbA_{1c}) into controlled T2DM where the HbA_{1c} is maintained at ≤7%, and uncontrolled T2DM where the HbA_{1c} level exceeds 7% (6,7). Of note, several studies reported that large percentage (30-83%) of diabetic patients are unable to control their blood glucose levels despite treatment with different glucose-lowering medications (8-10). Poor glycemic control among T2DM increases the risk of development of diabetic complications irrespective of the main treatment. Therefore, glycemic control is considered the main therapeutic objective to improve the quality of life and to prevent organ damage in diabetic patients.

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Chronic systemic inflammation and disordered abundance of various growth factors are a prominent feature of T2DM and are suggested to play a role in the pathogenesis and progression of diabetes-related complications (11,12). Importantly, several growth factors such as the epidermal growth factor (EGF) are involved in numerous biological processes, such as cell proliferation, differentiation, migration and wound healing, are also involved in the pancreatic β cell function, development, glucose regulation and insulin secretion (13,14). Moreover, EGF was shown to exert anti-inflammatory effects on the pancreas in animal models of pancreatitis (15). EGF is produced in different tissues such as the pancreas, kidney and the digestive system, and its circulating level is reduced in diabetic patients and animal models of diabetes (13,16). Therefore, the intricate relationship between poor glycemic control, altered EGF levels, and insulin resistance creates a complex feedback loop of metabolic dysregulation that has not been fully elucidated. This gap in understanding highlights the need for further investigation to uncover the underlying mechanisms and their potential implications for more effective management of T2DM.

This study aimed to estimate the relative abundance of various inflammatory markers and growth factors in patients with controlled and uncontrolled T2DM in Jordan, to assess their correlation with the glycemic status and insulin resistance and to evaluate their value in predicting disease progression. This research is crucial for the Jordanian population, where T2DM prevalence is rising, and the progression of insulin resistance and glycemic dysregulation often appears inevitable. Elucidating this relationship could unveil novel therapeutic strategies to mitigate diabetic complications, addressing the distinct metabolic factors influencing diabetes in the Jordanian population.

Materials and methods

Study design. An observational case-control design was used in the present study. Ethical approval to recruit subjects to participate in the study was received from The Institutional Review Boards of the Jordan University of Science and Technology (approval no. 7/114/2018; Irbid, Jordan). All study subjects were informed about the procedures and data collection prior to the start of study. Signed written consent forms were obtained from all participants followed by whole blood sampling. Recruitment of study subjects took place between December 2018 and December 2019 at the endocrinology clinics at King Abdullah University Hospital (KAUH), a tertiary hospital affiliated with Jordan University of Science and Technology in the northern part of Jordan. All research procedures were conducted following the Principle of Good Clinical Practice and the Declaration of Helsinki.

Study population. A total of 200 male and 200 female diabetic patients were invited to participate in the study. Inclusion criteria were as follows: Age >18 years-old and previous diagnosis with T2DM, whereas exclusion criteria were: patients with T1DM, pregnant women, patients with malignancies, Cushing's syndrome, or thyroid dysfunction. Subjects receiving insulin were also excluded from the study since it could affect HOMA-IR measurements. Of the invited patients, 240 agreed to participate and were pre-allocated for the study. Cases were defined as patients with uncontrolled T2DM (HbA1c >7%, n=120) and 1:1 matched them with controls (HbA1c ≤7%, n=120) based on age (±5 years), sex, body mass index (BMI) (±2 kg/m^2), and treatment type (metformin monotherapy). After data collection, outliers were identified using the z-score method, where values exceeding a z-score of 3.3 were flagged for further examination. These were then confirmed through box-plot analysis and outliers' analysis in SPSS. Since all statistical tests used in the present study require data normality, outliers that compromised the normal distribution were removed to ensure an acceptable level of skewness. Patients with missing data or significant outliers were excluded from analysis, and eventually 105 patients with uncontrolled T2DM and 110 patients with controlled T2DM were included in the final analysis.

Anthropometric measurements. Patients who met the eligibility criteria were interviewed by the attending physician during their visit to the clinic, and relevant information was obtained into a structured data collection sheet. Medical history, family history, height (cm), and weight (kg), waist circumference (cm) of the patients were recorded during their visit. BMI was calculated based on the aforementioned measurements using the following equation: BMI=weight (kg)/height^2 (m^2). The age of the patient was recovered from the patients' electronic medical records.

Blood sampling and handling. A certified phlebotomist withdrew two blood samples (5 ml each) by venipuncture from each participant after a 12-h fast. One blood sample was collected into an ethylene-diamine-tetra-acetic acid (EDTA) tube (AFCO) and then kept at 4˚C to be used for HbA_{1c} measurement. The second sample was collected into a plain tube containing a gel clot activator (AFCO) and allowed to clot at room temperature. This sample was then centrifuged at 4˚C at 4,000 x g for 5 min to separate the serum. The serum was immediately aliquoted into smaller volumes to prevent degradation due to repeated freeze-thaw cycles. The aliquoted serum samples were then deep-frozen at -80˚C and subsequently used for biochemical measurements, including glucose, total cholesterol, triglycerides and cytokine levels.

HbA_{1c} measurement. The blood samples stored in EDTA tubes were used to measure HbA_{1c} levels using an automated cobas c 513 analyzer system which is an *in vitro* diagnostic test system designed to quantitatively determine the percentage of HbA_{1c} in human capillary and venous whole blood by photometric transmission measurement (Roche Diagnostics) at the laboratories of KAUH. Patients having HbA_{1c} level >7% were considered to have an uncontrolled T2DM, whereas patients having HbA_{1c} ≤7% were considered to have controlled T2DM.

Biochemical measurements. To evaluate differential cytokines and growth factors level in the serum samples, a human cytokine antibody membrane array targeting 42 proteins (cat. no. ab133997; Abcam) was used according to the manufacturer's protocol. Briefly, 20 μl of serum samples from each patient of controlled T2DM and uncontrolled T2DM groups were pooled into two pools (labeled controlled

and uncontrolled T2DM), then 1 ml of each pool was incubated overnight into a designated well at 4˚C containing the membrane array. After 24 h, serum samples were aspirated, and the membranes were washed with wash buffer and then incubated with 1 ml of biotin-conjugated anti-cytokines overnight at 4˚C. Then the biotin-conjugated anti-cytokines were aspirated and washed. Finally, 2 ml of horseradish peroxidase-conjugated streptavidin was added into each well and incubated overnight at 4˚C; then it was aspirated, and the membranes were washed again by the previously described method. Detection buffer was used to develop a chemiluminescent signal and the C-DIGIT blot scanner (LI-COR Biosciences) was used to detect the signal intensity. The blots were visually assessed for relative differences in the abundance of inflammatory mediators and growth factors between the controlled and uncontrolled T2DM groups. The EGF, CXCL5/epithelial cell-derived neutrophil-activating peptide (ENA-78), stem cell factor (SCF), and C-X-C motif chemokine ligand 1 (CXCL1/GRO alpha, GRO-α) showed relative difference between groups, therefore their serum levels were evaluated using a commercially available DuoSet ELISA kits purchased from R&D Systems, Inc. The ELISA kits used in the present study are based on the solid-phase sandwich ELISA technique. The Human EGF ELISA (cat. no. DY236) has an assay range of 3.9-250 pg/ml. The Human CXCL5/ENA-78 ELISA (cat. no. DY254) provides an assay range of 15.6-1,000 pg/ml. The Human SCF ELISA (cat. no. DY255) and the Human CXCL1/GRO alpha ELISA (cat. no. DY275) both have assay ranges of 31.2-2,000 pg/ml. In addition, serum insulin levels were assessed by a solid phase sandwich Quantikine ELISA kit (cat. no. DINS00; R&D Systems, Inc.; sensitivity: 2.15 pmol/l, assay range: 15.6-500 pmol/l). Moreover, serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 alpha (IL-1α) and interleukin-1 beta (IL-1β) were measured using ELISA kits from R&D Systems, Inc. The TNF-α assay (cat. no. DTA00D) had a sensitivity of 6.23 pg/ml and a measurement range of 15.6-1,000 pg/ml. The IL-1α assay (cat. no. DLA50) had a sensitivity of 1 pg/ml and a range of 3.9-250 pg/ml. Similarly, the IL-1β assay (cat. no. DLB50) had a sensitivity of 1 pg/ml with the same assay range of 3.9-250 pg/ml. Briefly, the ELISA was carried out in duplicates of 100 μl aliquots of the serum samples, diluted accordingly to comply with the detection range of the relevant assay. A total of 100 μl of the standard solution was added to the wells of a 96-well plate pre-coated with a monoclonal antibody. Following the appropriate incubation period, the plate was washed, and an enzyme-labeled antibody, supplied as part of the assay kit, was added, followed by the substrate. The reaction was stopped by adding the stop solution after the development of color. The optical density of each well was determined by measuring the absorbance at 450 nm using an absorption spectrophotometer (Bio-Tek Instruments, Inc.). Furthermore, the serum samples were submitted to the laboratories of KAUH to measure glucose, total cholesterol and triglyceride levels using a high throughput automated analyzer system (cobas® modular analyzer series; Roche Diagnostics). Insulin resistance score (HOMA-IR) was calculated according to the formula: Fasting serum insulin (μU/l) x fasting serum glucose (nmol/l)/22.5.

Statistical analysis. Statistical analyses were performed using the IBM SPSS software ver. 22.0 (IBM Corp.). The unpaired Student's t-test was used to test for significant differences in serum cytokine levels, age, BMI, weight circumference total cholesterol, triglyceride and glucose levels between patients with controlled and uncontrolled T2DM. The binary logistic model was used to predict the likelihood of being uncontrolled diabetic based on EGF and HOMA-IR; factors that were significant on the t-test. A P-value of <0.05 was used as a cut-off for significance. Additionally, a Pearson's product-moment correlation was run to assess the relationship between serum EGF levels, HOMA-IR, fasting blood sugar (FBS) and HbA_{1c} indicative of glycemic control using SigmaPlot 12 software (Systat Software, Inc.).

Results

Patient characteristics and biochemical profile. During the course of the present study, 110 patients with controlled T2DM and 105 patients with uncontrolled T2DM were eligible to participate in the study. A schematic diagram that summarizes recruitment of study subjects is demonstrated in Fig. 1. Those patients were previously diagnosed with T2DM by a specialist endocrinologist according to the American Diabetes Association guidelines.

The percentage of women was 53% among controlled T2DM and 54% among uncontrolled T2DM with no significant differences existing in sex distribution between controlled and uncontrolled T2DM groups. The biochemical profile of the patients showed that patients with uncontrolled T2DM had significantly higher levels of fasting blood sugar (FBS), higher HbA_{1c} percentage, higher serum insulin levels, HOMA-IR score, and serum triglycerides level, with no difference in cholesterol levels between study groups.

The relative abundance of various cytokines and growth factor levels was evaluated using a human antibody cytokine membrane array. Surprisingly, a large number of prominent cytokines such as the interleukins were not detected on the array, and this was confirmed with ELISA (Table SI). However, certain chemokines and growth factors such as EGF, ENA-78, GRO-α and SCF were detected and revealed a visual difference in the cytokine array and therefore the results were assessed with ELISA (Fig. 2). Compared with the controlled T2DM subjects, the uncontrolled group had significantly lower EGF levels (95.9±82.7 vs. 158.77±111.7 pg/ml, P=0.002), representing ~40% reduction, while GRO-α showed a tendency for significant increase in the uncontrolled T2DM subjects (P=0.06). However, ENA-78 and SCF levels were not significantly different between study groups. The baseline characteristics of the study subjects and their biochemical profile are included in Table I.

Association between EGF, insulin resistance and glycemic control in T2DM. The binary logistic model was used to predict the likelihood of being uncontrolled diabetic based on EGF and HOMA-IR; factors that were significant on the t-test. After controlling for age, sex, and BMI both factors were statistically associated with diabetes control; higher HOMA-IR scores and lower EGF levels predicted uncontrolled diabetes, as shown in Table II. The underlying assumptions of the binary logistic

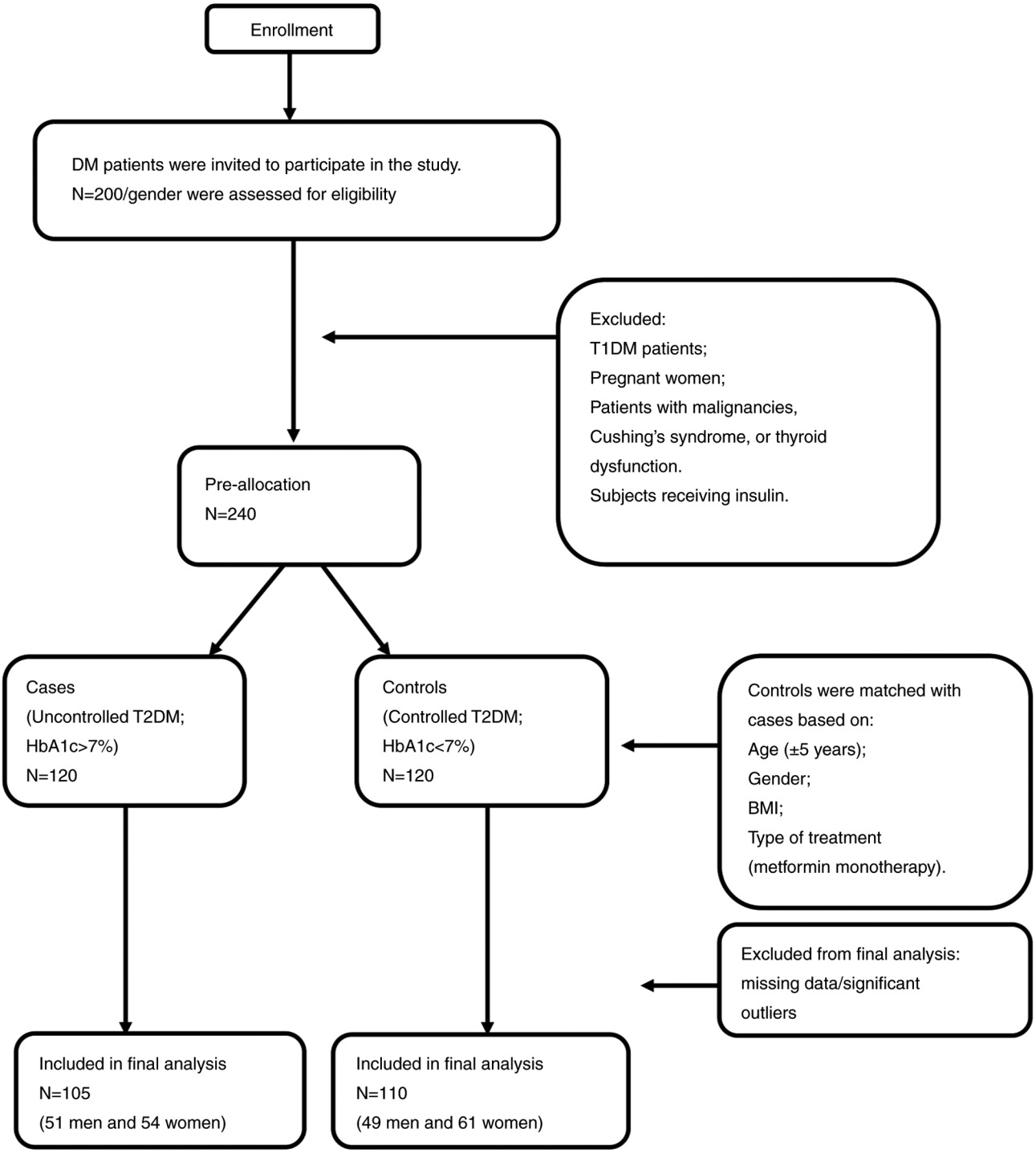
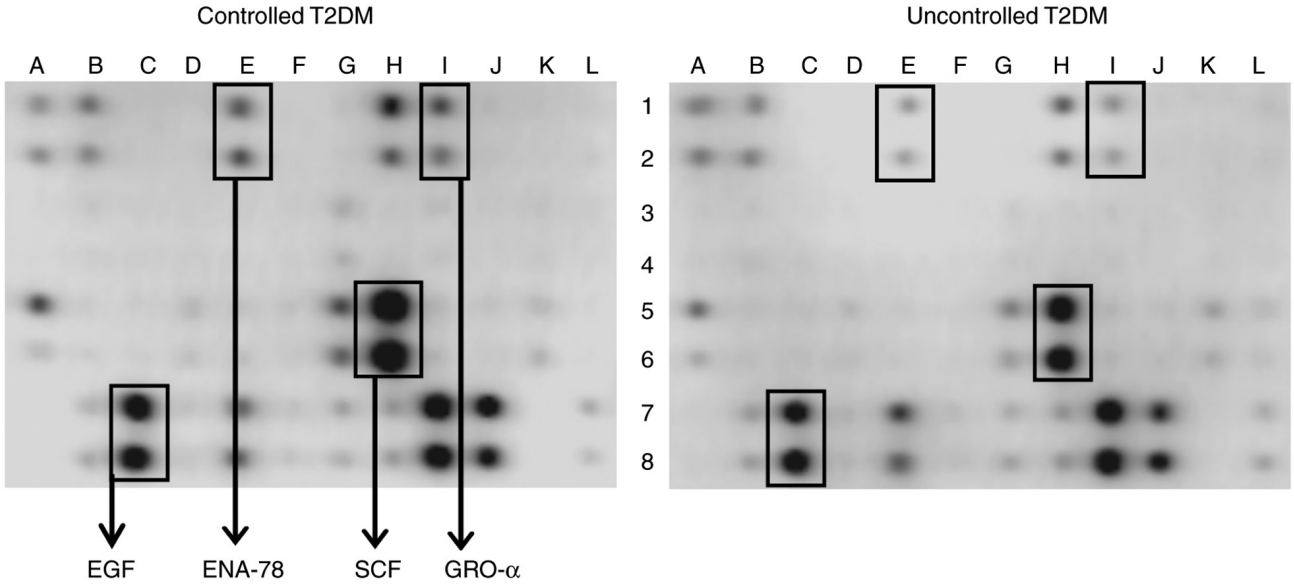


Figure 1. Patient recruitment process for the study. Schematic diagram illustrating the patient recruitment process for the study. The diagram outlines the selection criteria, the number of participants at each stage, and the grouping of patients into controlled and uncontrolled T2DM cohorts. T2DM, type 2 diabetes mellitus.

regression were the normality of data distribution, independence of observations and errors, and the absence of extremely high correlation between any 2 predictors that could suspect collinearity was assessed using variance inflation factors. The goodness-of-fit of the model was evaluated using the Hosmer-Lemeshow test, which assesses how well the observed data fit the model. A non-significant P-value (>0.05) from this test indicates that the model's predicted probabilities align well with the observed outcomes, suggesting a favorable fit.

Additionally, a Pearson's product-moment correlation was run to assess the relationship between serum EGF levels, HOMA-IR and HbA_{1c} indicative of glycemic control. There was a statistically significant positive correlation between HOMA-IR and HBA1c (R=0.321; P<0.001), significant negative correlation between EGF and HOMA-IR (R=-0.14; P=0.03), negative correlation between EGF and HbA_{1c}, (R=-0.248, P<0.001) and a negative correlation between EGF and FBS (R=-0.242, P<0.001) (Fig. 3).

A



B

	A	B	C	D	E	F	G	H	I	G	K	L
1	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1 β
2	Pos	Pos	Neg	Neg	ENA-78	GCSF	GM-CSF	GRO	GRO-α	I-309	IL-1α	IL-1 β
3	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12 p40/p70	IL-13	IL-15	IFN-γ
4	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12 p40/p70	IL-13	IL-15	IFN-γ
5	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	IFN-γ	MIP-1 δ	RANTES	SCF	TRAC	TGF- β1
6	MCP-1	MCP-2	MCP-3	MCSF	MDC	MIG	IFN-γ	MIP-1 δ	RANTES	SCF	TRAC	TGF- β1
7	TNF-α	TNF-β	EGF	IGF-I	Angiogenin	Oncostatin M	Thrombopoietin	VEGF	PDGF BB	Leptin	Neg	Pos
8	TNF-α	TNF-β	EGF	IGF-I	Angiogenin	Oncostatin M	Thrombopoietin	VEGF	PDGF BB	Leptin	Neg	Pos

Figure 2. Relative abundance of different inflammatory mediators and growth factors in patients with controlled and uncontrolled T2DM. (A) To evaluate differential cytokines and growth factors level in the serum samples, a human cytokine antibody membrane array targeting 42 proteins was used according to the manufacturer's protocol. Briefly, 20 μl of serum samples from each patient of controlled T2DM and uncontrolled T2DM groups were pooled into two pools (labeled controlled and uncontrolled T2DM), then 1 ml of each pool was incubated overnight into a designated well at 4˚C containing the membrane array. A visual comparison between the two arrays performed by two independent investigators revealed that five cytokines were different between groups: The EGF, ENA-78, SCF and GRO-α. (B) List of cytokines and chemokines represented by the antibody array membrane. T2DM, type 2 diabetes mellitus; EGF, epidermal growth factor; ENA-78, CXCL5/epithelial cell-derived neutrophil-activating peptide; SCF, stem cell factor; GRO-α, C-X-C motif chemokine ligand 1.

Discussion

Despite the scientific advances in the management of T2DM, it remains a major threat to public health globally due to its epidemic nature and its effect on the wellbeing of the patients (17,18). It is well known that T2DM increases the risk of developing various health problems such as cardiovascular diseases, dyslipidemia, neuropathy, loss of vision, nephropathy, diabetic foot ulcers and leg amputations. The hyperglycemic state and disturbed endocrine milieu are usually associated with increased serum levels of various inflammatory markers and growth factors, which are considered to mediate these diabetic complications (19,20). Moreover, T2DM is considered as a progressive disease, that can lead over time to irreversible complications due to chronic hyperglycemia, oxidative stress, metabolic derangements and glucolipotoxicity (21,22).

HbA_{1c}, which reflects the cumulative glycemic history of the preceding 2-3 months, is considered as an indicator of overall glycemic control and the potential long-term diabetic complications. Several studies reported increasing loss of glycemic control over time in patients with T2DM, despite the use of various glucose-lowering medications such as metformin and sulfonylureas (10,23,24). Therefore, regular follow up of the patients, adopting an active lifestyle and exercise to maintain adequate glycemic control in T2DM is crucial to reduce the mortality and morbidity of diabetes.

The association between serum levels of inflammatory markers and the glycemic state in T2DM was not previously investigated in diabetic patients in Jordan. In the current study, the role of glycemic control on the relative abundance of inflammatory markers and growth factors was investigated in patients with T2DM sub-grouped into age-sex-, and BMI-matched controlled diabetic group (HbA_{1c} ≤7%) and

Table I. Baseline characteristics of controlled and uncontrolled diabetic participants.

Characteristics	Controlled T2DM, n=110	Uncontrolled T2DM n=105	P-value
Sex			0.557
Males, n (%)	49 (49)	51 (51)	
Females, n (%)	61 (53)	54 (47)	
Age (years)	60.12±9.27	60.89±10.82	0.56
Weight (Kg)	83.94±16.54	83.74±15.46	0.71
Height (cm)	166.3±8.6	167.2±8.7	0.79
Waist circumference (cm)	106.6±11.3	108.8±12.43	0.46
Body mass index	30.3±5.47	29.9±5.01	0.63
Glycated hemoglobin	6.25±0.46	8.97±1.41	<0.001
Glucose (mg/dl)	138.2±38.86	202.8±68.56	<0.001
Cholesterol (mg/dl)	207.8±58.39	211.1±56.7	0.89
Triglyceride (mg/dl)	140.32±75.35	179.46±131.13	0.025
GRO-α (pg/ml)	194.98+81.9	229.1±123.47	0.06
EGF (pg/ml)	158.77±111.7	95.9±82.7	0.002
ENA-78 (pg/ml)	489.13±326.31	548.4±318.27	0.99
SCF (pg/ml)	66±26.57	61.3±25.1	0.33
HOMA-IR	9.6±8.2	16±10.5	0.001
Insulin (pmol/l)	163.37±123.68	209.22±182.68	0.02

All continuous variables are presented as the mean ± standard deviation (Mean ± SD)

Data are presented as the mean ± standard deviation. The P-values were calculated by Student's t-test except for sex distribution which was calculated using Pearson's chi-square. T2DM, type 2 diabetes mellitus; HOMA-IR, homeostatic model assessment insulin resistance; GRO-α, C-X-C motif chemokine ligand 1; EGF, epidermal growth factor; ENA-78, CXCL5/epithelial cell-derived neutrophil-activating peptide; SCF, stem cell factor.

Table II. Binary logistic regression model predicting the likelihood of uncontrolled diabetes based on EGF and HOMA-IR levels, adjusted for age, sex and BMI.

							95% CI for odds ratio	
	B	SE	Wald	df	P	Odds ratio	Lower	Upper
EGF	-0.006	0.002	13.747	1	0.000	0.994	0.991	0.997
HOMA-IR	0.073	0.018	16.747	1	0.000	1.075	1.039	1.114
Constant	-0.262	0.321	0.666	1	0.414	0.770		

The binary logistic model was used to predict the likelihood of being uncontrolled diabetic based on EGF and HOMA-IR; factors that were significant on the t-test. After controlling for age, sex and BMI both factors were statistically associated with diabetes control; higher HOMA-IR scores and lower EGF levels predicted uncontrolled diabetes. EGF, epidermal growth factor; HOMA-IR, homeostatic model assessment insulin resistance; BMI, body mass index; CI, confidence interval.

uncontrolled diabetic group (HbA_{1c} >7%) using an observational case-control study design. It was found that patients with uncontrolled T2DM had significantly lower levels of serum EGF compared with patients with controlled T2DM. Moreover, patients with uncontrolled T2DM had significantly higher levels of FBS, serum triglycerides, serum insulin, HOMA-IR and a slight increase in GRO-α, compared with patients with controlled T2DM. Furthermore, the binary logistic regression demonstrated that higher HOMA-IR scores and lower EGF levels predicted an increased likelihood of being an uncontrolled diabetic.

The negative correlation between EGF and HbA_{1c} in these study subjects indicates that EGF may exert direct effects or is involved in the regulation of glucose homeostasis and overall glycemic control in the body. EGF, a small transmembrane polypeptide secreted by a variety of tissues such as the loop of Henle, distal convoluted tubule in the kidney, salivary glands and duodenum and the pancreas, is a potent mitogen and regulator of a wide range of cellular processes (25,26). After binding to its receptor (EGFR), a member of the tyrosine kinase receptor superfamily, it activates various signaling pathways that regulate cellular proliferation, differentiation, secretion

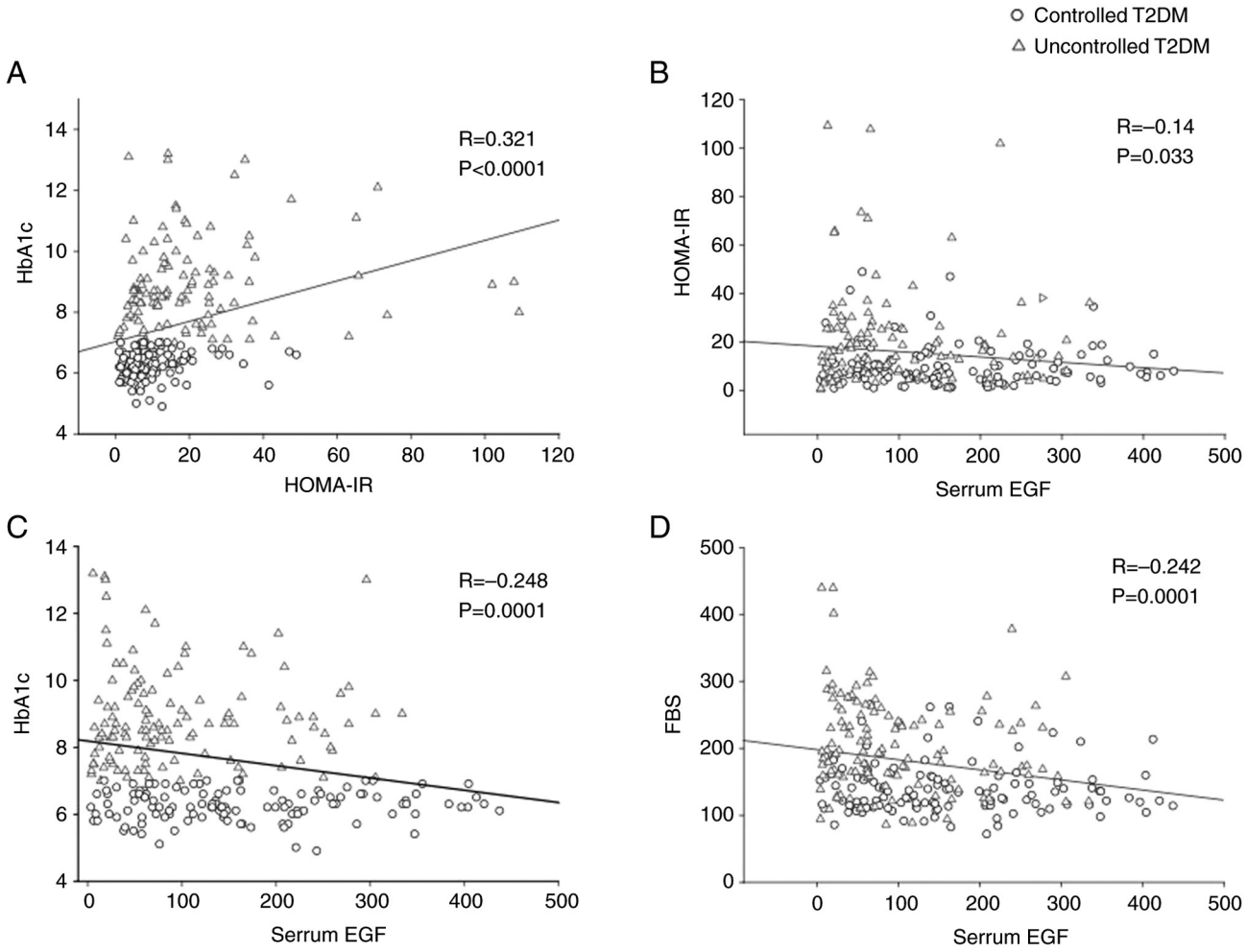


Figure 3. Correlations between serum EGF levels and various metabolic parameters in controlled and uncontrolled T2DM subjects. Scatter plots illustrating the relationships between serum EGF levels and metabolic parameters in controlled (black circles) and uncontrolled (red triangles) T2DM subjects. (A) Correlation between HbA1c and HOMA-IR (R=0.321; P<0.0001). (B) Correlation between serum EGF levels and HOMA-IR (R=-0.14; P=0.033). (C) Correlation between serum EGF levels and HbA_{1c} (R=-0.248, P=0.0001). (D) Correlation between serum EGF levels and FBS (R=-0.242; P=0.0001). The uncontrolled T2DM group exhibits significantly lower EGF levels compared with the controlled T2DM group, indicating a negative association between serum EGF levels and glycemic control parameters. EGF, epidermal growth factor; T2DM, type 2 diabetes mellitus; HbA_{1c}, glycated hemoglobin; HOMA-IR, homeostatic model assessment insulin resistance; FBS, fasting blood sugar.

and apoptosis. EGFR activation results in the activation of phosphatidylinositol pathway through the activation of protein kinase C and inositol (1,4,5)-trisphosphate [Ins(1,4,5)P3] as well as by increasing the intracellular cytoplasmic calcium concentration (27).

In fact, EGF is proposed to have a role in the development of the pancreas, pancreatic β cells regeneration, insulin and glucagon secretion and glucose homeostasis (28). Moreover, EGFR is expressed throughout the human fetal pancreas, and its absence in mice resulted in abnormal pancreatic islets development (14). Additionally, it has been shown that EGF has a vital role in generating β-cells, regulation of their insulin content, and maintenance of their mass through stimulation of the anti-apoptotic protein survivin (29,30). Moreover, studies have identified that EGF acts as secretagogue that lowers plasma glucose levels in normal and diabetic mice via a Ca^{2+} influx-and PLD2-dependent mechanism by regulation of glucose transporters' activity and expression (13). Interestingly, EGF deficiency is associated with diabetes mellitus in animals, where EGF or EGFR levels are decreased in various organs or fluids, such as the liver, the submandibular gland, plasma and milk (31). Furthermore, reduced EGF levels are associated with chronicity and severity of diabetic foot ulcers, therefore topical and intralesional EGF administration have been implemented to improve and speed ulcer healing (32,33).

Previous research showed that EGF A61G polymorphism (rs4444903) is a genetic variation associated with various types of cancer (34). This single nucleotide change shows significant differences in frequency across ethnic groups (35). Interestingly, research by Trimal *et al* (36) revealed that EGF A61G gene single nucleotide polymorphism (SNP) was associated with increased risk of developing T2DM in Indian population, and low EGF mRNA expression in T2DM when compared with healthy controls. The aforementioned study demonstrated that individuals with the A/G genotype have a substantially higher risk (~4-fold) of developing T2DM compared with those with the G/G genotype. The study also found that the 'A' allele was more common in patients with T2DM than in healthy controls, suggesting it increases the risk of T2DM by 1.91-fold compared with the 'G' allele.

Additionally, both AG and AA genotypes were linked to notably reduced EGF mRNA expression levels when compared with the GG genotype (36). Furthermore, reduced concentrations of EGF have been observed in other conditions such as diabetic nephropathy, IgA nephropathy, adult polycystic kidney disease and children with chronic renal failure (37).

EGF's actions extend to diverse molecular and biochemical pathways at both local and systemic levels such as neural maturation, myelination, immunomodulation, inhibition of inflammatory mediators, reduction-oxidation balance, decreased toxic glycation products, and intestinal development and barrier function through regulation of tight junction protein expression, autophagy, and apoptosis of epithelial cells (38-40).

Kawaguchi *et al* (41) reported a significant positive correlation between urinary EGF excretion and HbA_{1c} levels in diabetic patients with inadequate glycemic control when the HbA_{1c} level is >8%, and lack of correlation in these parameters when the HbA_{1c} is <8%, possibly due to increased glomerular filtration rate due to hyperglycemia. On the other hand, previous studies in diabetic patients and animal models of diabetes reported reduced urinary EGF expression and secretion in case of diabetes reflecting diabetes-induced renal tubular injury (42). Notably, several studies demonstrated that urinary EGF (uEGF) levels decrease across various kidney diseases, including diabetic nephropathy. Importantly, uEGF has been validated as a predictor of kidney damage in patients with type 2 diabetes and as a non-invasive prognostic biomarker for chronic kidney disease even in the absence of albuminuria (43). Therefore, it is plausible to postulate that loss of glycemic control and elevated HbA_{1c} level reduces serum EGF levels possibly through affecting renal EGF handling and expression, and this may lead to further decline in β cell mass and further deterioration in glucose homeostasis.

The positive correlation between HOMA-IR and HbA_{1c} in the present study is in accordance with previous published studies, emphasizing the role of glycemic control on the status of insulin resistance, which has been postulated to be an important and independent risk factor for the development of cardiovascular diseases, retinopathy and diabetic foot (19,44). Although the glucose clamp technique and HOMA-IR are the standard methods to measure insulin resistance, the present study revealed that HbA_{1c} can also be a valuable assist to predict insulin resistance and potential long-term complications (45-47). The current study identified a significant negative correlation between serum EGF levels and HbA1c, indicating that higher HbA1c levels are associated with lower serum EGF levels, which may impair wound healing and vascular health due to reduced cellular repair and endothelial function. Additionally, a significant positive correlation between HOMA-IR and HbA1c suggests that higher insulin resistance correlates with poorer glycemic control, exacerbating metabolic dysfunction and beta-cell stress. Moreover, it was hypothesized that the observed 40% reduction in serum EGF levels in patients with uncontrolled T2DM has significant biological implications, particularly in terms of wound healing, vascular health and metabolic regulation. The potential mechanisms behind this finding include hyperglycemia-induced oxidative stress, chronic inflammation, disrupted insulin signaling, renal function impairment and the impact of advanced glycation end products. These findings highlight the impact of chronic hyperglycemia on growth factor levels and the central role of insulin resistance in T2DM, emphasizing the need for comprehensive management strategies that address both glycemic control and insulin sensitivity to mitigate complications and improve patient outcomes. Understanding these mechanisms can provide insights into the pathophysiology of T2DM and inform targeted therapeutic strategies to mitigate these effects.

Despite previous studies indicating increased serum levels of various cytokines and inflammatory markers in diabetic patients, a lack of detectable cytokine expression of the majority of inflammatory mediators such IL-1α, IL-1β and TNF-α was observed in this cohort of patients, with both controlled and uncontrolled T2DM (Table SI). Possible explanations of this intriguing observation include the fact that those patients were treated with metformin, which is reported to have anti-inflammatory effects which could potentially normalize interleukin and TNF-α levels across different patient groups (48). This uniformity in medication might have mitigated any expected variations in inflammatory markers, thereby contributing to the observed lack of significant differences. Additionally, the relatively moderate BMI of the cohort, with an average ~30, along with the ethnic background of the patients, may have further contributed to the absence of significant variations in cytokine levels.

The present study has several limitations that should be acknowledged. Firstly, the cross-sectional design restricts our ability to establish causal relationships between glycemic control, serum EGF levels and insulin resistance; while the observed associations offer valuable insights, they do not confirm causation. Additionally, data on some variables were not collected, such as the duration of diabetes, stages of diabetic nephropathy, or other comorbid conditions, which may have an influence on the study outcomes. Moreover, the sample size may limit the generalizability of the present findings to the broader Jordanian population, especially across its diverse ethnic and demographic groups. The results of the current study were adjusted for key well-established demographic confounding factors known to influence glycemic control and associated health outcomes. Focus was addressed on these global confounders as they are among the most significant determinants in this context. It is acknowledged that other factors could also potentially influence the outcomes; however, based on the present study design and the available data, it was determined that age, sex and BMI were the most critical factors to control for to ensure the validity of our findings. Moreover, while the statistical methods were appropriate and justified for the present study, it is acknowledged that they do have limitations. Specifically, these methods may not fully capture complex interactions between variables. Nonetheless, they were effective in exploring the associations between glycemic control, serum EGF levels and insulin resistance. Future studies with longitudinal designs, larger sample sizes, and more comprehensive data collection are necessary to address these limitations and provide a clearer understanding of the complex interactions involved in EGF's role in insulin resistance and glycemic control. Such studies could provide valuable insights into how changes in glycemic control over time impact EGF levels and, conversely, how EGF levels might influence the progression of diabetes and its complications.

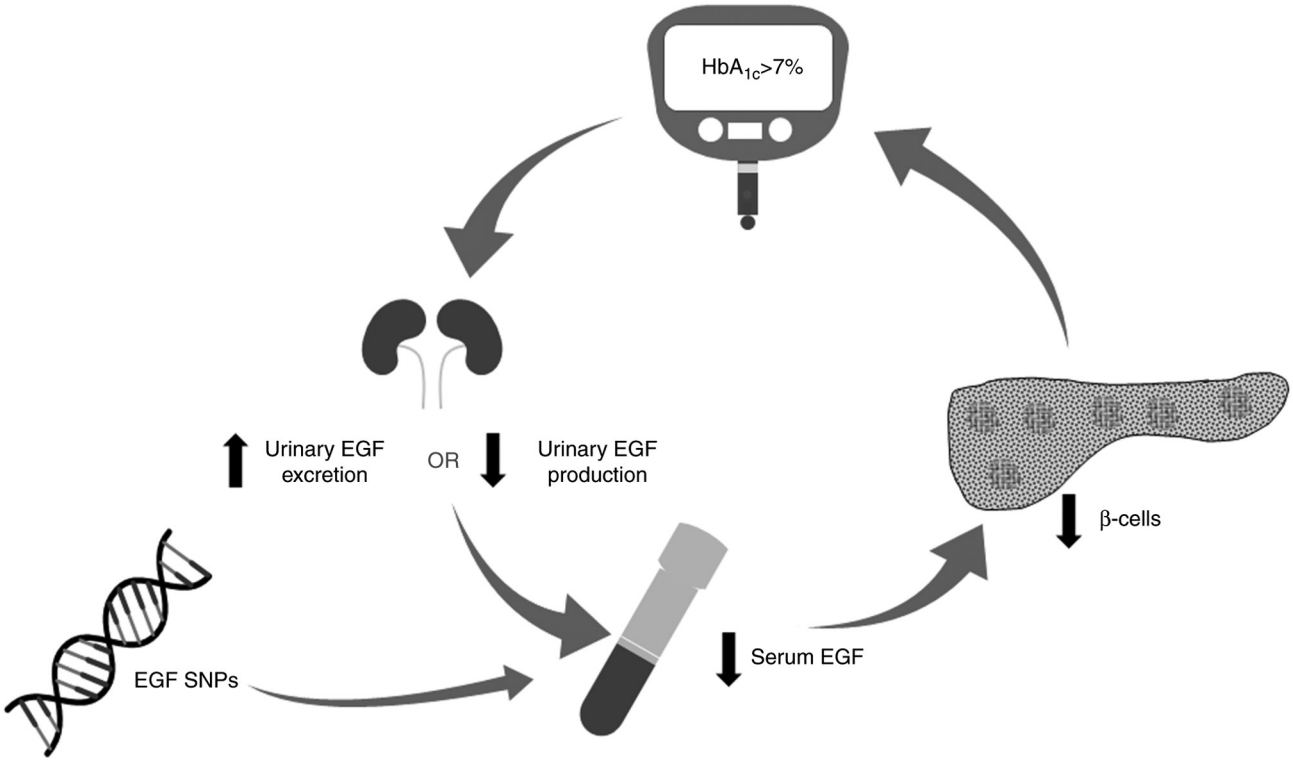


Figure 4. A proposed integrative model of possible role of EGF in regulating β-cell function and glycemic control. It was hypothesized that loss of glycemic control in patients with type 2 diabetes mellitus may lead to increased urinary EGF excretion due to hyperfiltration through the kidneys, or reduced expression of EGF in the kidneys, leading to reduced serum EGF levels, which in turn causes further deterioration of β-cell function and insulin secretion. Furthermore, variation in the genetic makeup of the patients such as SNPs in EGF gene may lead to reduced EGF levels. EGF, epidermal growth factor; HbA_{1c}, glycated hemoglobin; SNPs, single nucleotide polymorphisms.

In addition, since multiple statistical tests were conducted in the present study, it is important to acknowledge that multiplicity could occur, increasing the risk of Type I errors, or false positives. While the results were carefully interpreted, the potential for inflated significance due to the number of comparisons cannot be fully excluded. Future studies with larger sample sizes and more stringent correction methods, such as Bonferroni adjustment or false discovery rate control, are recommended to confirm the findings and ensure the robustness of the observed associations.

In conclusion, the present study identified a significant negative association between serum EGF levels and glycemic control in patients with T2DM, suggesting that EGF may play a crucial role in the metabolic dysregulation observed in T2DM. This finding offers new insights into the pathophysiology of T2DM and underscores the potential use of serum EGF levels as a novel biomarker for assessing and monitoring glycemic control in clinical settings. Incorporating EGF into a panel of biomarkers could help identify patients with T2DM at risk of developing poor glycemic control, providing a more comprehensive approach to disease management and facilitating personalized treatment strategies. Furthermore, targeting EGF-related pathways may open up new therapeutic avenues to improve glycemic control and optimize the overall management of T2DM. An integrative model of the possible role of EGF in regulating insulin levels and glycemic control is proposed in Fig. 4, based on the findings from the present study and previous studies. It is hypothesized that loss of glycemic control in patients with T2DM decreases urinary EGF production or increases urinary EGF excretion due to hyperfiltration through the kidneys, ultimately leading to reduced serum EGF levels, which in turn causes further deterioration of β-cell function and insulin secretion. Furthermore, variation in the genetic makeup of the patients such as a SNPs in EGF gene may lead to reduced EGF levels. It is acknowledged that there is currently limited epidemiological data on EGF SNPs within the Jordanian population. As a result, any comparison between Jordanian and other populations, such as Indian individuals, carries a degree of speculation. The outcomes of the present study emphasize the importance of recognizing individuals with uncontrolled T2DM lies in the fact that early intervention may delay or prevent the progression of the disease and development of complications.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

AAD performed experimental design, acquired funding and wrote the manuscript. MAA and OA performed experimental design and data analysis. RAS conducted statistical analysis and wrote the manuscript. MA wrote the manuscript and performed data analysis. MK collected data. AK performed experimental design and interpretation of the data. AAD and OA confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of Jordan University of Science and Technology and King Abdullah University Hospital Institutional Review Board (approval no. 7/114/2018; Irbid Jordan), and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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