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TNF-α, fasting insulin, and microRNA-192 as biomarkers of disease severity and complications in pediatric type 1 diabetes mellitus

Amal Ahmed Mohamed [a], Noha Mohamed Mesbah [b], Asmaa Ramadan Abdel-Hamed [b], Kawkab Mtwaly [b], Eman El Sayed Mohamed Habib [c], May Mohsen Samy Selim [d], Salma Mohamed Saed [d], Mohamed Abdelfattah Ibrahim Saad [e], Heba Salah Shahin [f], Sherihan Hassan Elkady [g], Amira N. Ahmed [h,*], Nourhan Mekky [i], Weam Shaheen [j], Hoda H. Ahmed [k], Tarek Abdelkader Sallam [l], Nehal M. Khairy [m], Eman Alsayed Abouahmad [n]

[a] *Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, The General Organization for Teaching Hospitals and Institutes (GOTHI), Cairo, Egypt*
[b] *Biochemistry Department, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt*
[c] *Pediatric Department, National Nutrition Institute, Cairo, Egypt*
[d] *Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt*
[e] *Pediatric Department, Al Mataria Teaching Hospital, The General Organization for Teaching Hospitals and Institutes (GOTHI), Cairo, Egypt*
[f] *Anatomic Pathology Department, Faculty of Medicine, Cairo University, Cairo, Egypt*
[g] *Clinical and Chemical Pathology Department, Faculty of Dentistry, October University of Modern Sciences and Arts (MSA), 6th of October City, Egypt*
[h] *Clinical and Chemical Pathology Department, Medical Research and Clinical Studies Institute, National Research Centre, Giza, Egypt*
[i] *Basic Medical Science Department, Faculty of Dentistry, October University of Modern Sciences and Arts (MSA), 6th of October City, Egypt*
[j] *Environmental and Occupational Medicine Department, National Research Centre, Giza, Egypt*
[k] *Pediatric Department, Medical Research and Clinical Studies Institute, National Research Centre, Giza, Egypt*
[l] *Public Health Department, Military Medical Academy, Cairo, Egypt*
[m] *Department of Microbiology and Immunology, Egypt Drug Authority (EDA), Giza, Egypt*
[n] *Department of Clinical Pathology, Faculty of Medicine, Minia university, Minia, Egypt*

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ABSTRACT

Objective: To assess the diagnostic and clinical relevance of fasting insulin, TNF-α, and microRNA-192 in relation to diabetes complications in pediatric T1DM, highlighting the complex interplay between metabolic, inflammatory, and molecular factors.

Patients and methods: This prospective cross-sectional case-control study compared 100 children with established type 1 diabetes mellitus (T1DM) to 100 age-matched healthy controls (total cohort: 98 males, 102 females). Serum levels of TNF-α, fasting insulin, and microRNA-192 expression were quantified. Multiplicity was adjusted using the Benjamini–Hochberg false discovery rate (FDR), and independent biomarker associations with a composite complication endpoint (eye, neurological, nephropathic, or cardiac) were evaluated using multivariable binary logistic regression adjusted for age, sex, BMI, and disease duration.

Result: Continuous variables deviated significantly from normality and were analyzed non-parametrically. T1DM patients demonstrated significantly higher median fasting insulin [11.0 (IQR 8.2–13.0) vs. 3.60 (IQR 3.00–4.10) mIU/L; $q<0.001$] and modestly elevated TNF-α [31.0 (IQR 23.0–45.0) vs. 28.0 (IQR 23.0–30.0) pg/mL; $q=0.006$], alongside lower serum microRNA-192 expression [0.74 (IQR 0.56–0.88) vs. 1.07 (IQR 0.54–1.74); $q<0.001$]. Corrected ROC analysis demonstrated excellent discrimination for fasting insulin (AUC=0.972, sensitivity=97%, specificity=98%), but modest to limited utility for microRNA-192 (AUC=0.683) and TNF-α (AUC=0.612, sensitivity=50%, specificity=91%). In multivariable logistic regression, only fasting insulin

* Correspondence to: A.N. Ahmed, Department of Clinical and Chemical Pathology, National Research Centre, Cairo, Egypt.
E-mail addresses: amalahmedhcp@yahoo.com (A.A. Mohamed), noha_mesbah@pharm.suez.edu.eg (N.M. Mesbah), asmaa.ramdan@pharm.suez.edu.eg (A.R. Abdel-Hamed), katie.brhym@gmail.com (K. Mtwaly), eme.habib@yahoo.com (E.E.S.M. Habib), may.selim07@gmail.com (M.M.S. Selim), salma.saed@cu.edu.eg (S.M. Saed), mi151501@gmail.com (M.A.I. Saad), heba.shahin@kasralainy.edu.eg (H.S. Shahin), selkady@msa.edu.eg (S.H. Elkady), amira.koraitim@yahoo.com (A.N. Ahmed), nmekky@msa.edu.eg (N. Mekky), weamshaheen@gmail.com (W. Shaheen), hegazyhoda@yahoo.com (H.H. Ahmed), trksllm35@gmail.com (T.A. Sallam), nehal.khairy@su.edu.eg (N.M. Khairy), emanlab2003@yahoo.com (E.A. Abouahmad).

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(adjusted OR=2.38, q=0.003) and disease duration remained independently associated with diabetic complications, whereas the crude associations of TNF-α and microRNA-192 were no longer significant after confounding adjustment (q=0.202 and q=0.064, respectively).
Conclusion: Fasting insulin, reflecting overall metabolic burden rather than β-cell reserve, is independently associated with microvascular and macrovascular complications in pediatric T1DM. Conversely, circulating TNF-α and microRNA-192 do not independently predict complications once clinical factors like disease duration are controlled. These findings underscore the prominent role of metabolic stress over single inflammatory or epigenetic markers in early pediatric diabetes progression.

1. Introduction

Type 1 diabetes mellitus (T1DM) is one of the most common chronic endocrine disorders affecting children and adolescents.[1] Despite major advances in insulin therapy and glucose monitoring technologies, many pediatric patients develop microvascular complications at an early age, often progressing silently before clinical detection.[2]

Hyperglycemia, the hallmark of diabetes mellitus, contributes to microvascular injury through several interconnected biochemical pathways, including activation of the polyol pathway, increased formation of advanced glycation end products (AGEs), sustained activation of protein kinase C, and enhanced flux through the hexosamine pathway.[3] These mechanisms collectively promote oxidative stress, endothelial dysfunction, and chronic inflammation. Diabetic microvascular complications remain leading causes of acquired blindness, non-traumatic lower-extremity amputation, and end-stage renal disease worldwide.[4] Therefore, early identification of high-risk patients and timely intervention are essential to prevent irreversible organ damage.

However, glycemic control alone does not fully explain the heterogeneity in disease severity and complication risk among children with T1DM. Growing evidence indicates that metabolic stress, inflammatory mediators, and epigenetic mechanisms significantly contribute to the development and progression of diabetic complications.[5,6]

Tumor necrosis factor-alpha (TNF-α) is a pivotal pro-inflammatory cytokine implicated in endothelial dysfunction and insulin signaling impairment.[7] Chronic hyperglycemia induces a state of low-grade inflammation, in which TNF-α plays a central role by disrupting insulin receptor signaling, enhancing oxidative stress, and promoting vascular injury. Elevated circulating TNF-α levels have been reported in patients with diabetic nephropathy, where they correlate with albuminuria and declining renal function.[8] Increased concentrations have also been observed in diabetic peripheral neuropathy, further supporting its involvement in microvascular damage. Experimental data suggest that TNF-α activates nuclear factor kappa B (NF-κB)–mediated inflammatory pathways under hyperglycemic conditions, thereby accelerating vascular and metabolic deterioration. Collectively, these findings underscore the potential utility of TNF-α as a marker of inflammatory burden and disease progression in T1DM.

Although T1DM is classically characterized by autoimmune β-cell destruction and absolute insulin deficiency, increasing evidence supports the emergence of a "double diabetes" phenotype, particularly in children and adolescents with obesity or pubertal insulin resistance. In this context, features of insulin resistance coexist with autoimmune diabetes. Therefore, fasting circulating insulin was evaluated in the present study as a marker of metabolic dysregulation and disease severity rather than β-cell reserve [9,10]

Insulin resistance in T1DM has been associated with endothelial dysfunction, chronic low-grade inflammation, and enhanced oxidative stress, all of which contribute to vascular injury. Elevated circulating insulin levels may therefore serve as an indirect marker of underlying metabolic stress. Importantly, insulin resistance has been linked to the development and progression of both microvascular and macrovascular complications in patients with T1DM. These observations suggest that fasting insulin levels, within the framework of double diabetes, may provide insight into cardiometabolic risk and disease severity beyond glycemic control alone.[11] These observations suggest that fasting insulin may serve not only as a metabolic parameter but also as a potential indicator of complication risk in pediatric T1DM.[12]

MicroRNAs have emerged as important epigenetic regulators in metabolic diseases. It has been implicated in inflammatory signaling, fibrosis, and vascular injury; however, its role in pediatric T1DM and related complications remains insufficiently explored.[13]

These short non-coding RNA molecules influence key cellular processes such as inflammation, fibrosis, and metabolic stress, all of which are relevant to the development of diabetic complications. Among them, microRNA-192 has been studied in relation to diabetic kidney disease, where changes in its expression correlate with renal injury and progression of albuminuria. In several recent clinical and experimental reports, lower levels of circulating microRNA-192 have been associated with worsening kidney function, suggesting that this microRNA may reflect ongoing pathological changes in renal tissue.[14] Other studies have also linked altered microRNA profiles, including microRNA-192, to vascular dysfunction and chronic inflammation, both of which are central to complications such as retinopathy and atherosclerosis. Although results have varied depending on the biological sample and disease context, these findings highlight the potential of microRNA-192 and related microRNAs as markers of tissue damage and metabolic disturbance in diabetes.[15,16]

Accordingly, the present study aimed to evaluate TNF-α, fasting insulin, and microRNA-192 as potential biomarkers of disease severity and diabetic complications in children with T1DM.

2. Subjects and methods

2.1. Study design and ethical approval

This study is a prospective cross-sectional case–control investigation. Ethical approval was obtained from the National Nutrition Institute's Ethics Committee (approval number IN000154). All procedures were adhered to the Declaration of Helsinki, and written informed consent was obtained from the parents or legal guardians of all participants, after full discussion of the aim of the study.

The study compared children with established type 1 diabetes mellitus (T1DM) to age matched healthy controls. Sample size was determined based on a power calculation (80% power, $\alpha = 0.05$) to detect a moderate effect size in serum miR-192 levels, yielding 100 participants per group. Participants were recruited between January 2025 and December 2025.

2.2. Study participants

A total of 200 Egyptian children (98 males, 102 females) were enrolled and divided equally into two groups ($n = 100$ per group). T1DM patients ($n = 100$) are clinically diagnosed with T1DM and recruited from the outpatient clinic of the National Nutrition Institute. Healthy controls ($n = 100$) are age matched children without diabetes.

All participants underwent a structured interview to collect: Medical history, including T1DM onset, disease duration, and family history of diabetes. Also, lifestyle characteristics, including regular exercise (yes/no) and dietary pattern categorized as low–moderate carbohydrate,

moderate–high carbohydrate, high carbohydrate, or high protein. And, Anthropometric measurements, including body mass index (BMI) and waist circumference (WC). Information regarding partial clinical remission was not systematically recorded and was therefore not available for analysis. Pubertal status and Tanner staging were not routinely documented in the available clinical records and were therefore not included in the analysis. This may have resulted in residual confounding, as pubertal development can influence insulin sensitivity and BMI.

Clinical Assessment of Diabetic Complications: Microvascular and macrovascular complications were evaluated following standard clinical tracking and screening frameworks. Diabetic Retinopathy was screened via fundus photography or mydriatic ophthalmoscopy through dilated pupils, with staging evaluated by an experienced ophthalmologist according to international pediatric criteria. Diabetic Peripheral Neuropathy was assessed using clinical history and standard non-invasive physical examinations, including 10-g monofilament testing to evaluate loss of protective sensation, ankle reflex evaluation, and tactile or vibration sensation scoring using a 128-Hz tuning fork. Diabetic Nephropathy was defined based on the continuous monitoring of the estimated Glomerular Filtration Rate (e-GFR) calculated from serum creatinine using standard pediatric equations to track structural kidney function drops or hyperfiltration states. Given the lack of a standardized diagnostic endpoint for clinical macrovascular macro-angiopathy in young children, Cardiac Complications were defined as early cardiometabolic or vascular dysfunction endpoints; these were systematically assessed through clinical resting blood pressure measurements to screen for pediatric hypertension, standard fasting lipid panels (evaluating LDL-C, HDL-C, and triglycerides) to identify persistent dyslipidemia, and standard resting electrocardiography (ECG) to monitor for resting tachycardia or conduction disturbances.

2.3. Inclusion criteria

Confirmed T1DM diagnosis for cases and absence of diabetes for controls.

2.4. Exclusion criteria

Other chronic diseases (e.g., autoimmune disorders, liver or kidney disease) or acute infection. Recent hospitalization or medications affecting metabolic, inflammatory, or immune status.

2.5. Laboratory investigations

Sample Collection and Processing: participants fasted overnight (≥8 h, water allowed) prior to morning blood sampling. Blood samples were collected after an overnight fast and before administration of the morning rapid-acting insulin dose to minimize the influence of the most recent insulin injection. Because the insulin ELISA measures total circulating immunoreactive insulin, fasting insulin concentrations may still include residual exogenous basal insulin. Accordingly, fasting insulin was interpreted as a measure of circulating insulin exposure rather than endogenous insulin secretion. Approximately 7 mL of venous blood was collected from all participants under complete aseptic conditions and split as follows:
Hematological Analysis: part of the sample was anticoagulated with ethylene diamine tetra acetate "EDTA" for complete blood count, including total white blood cell (WBC) count, using the Phoenix NCC-3300 analyzer.
Rest of blood was allowed to clot for ≥30 min in sterile plain vacutainer, then centrifuged at 3000 rpm and sera were separated immediately for analysis of:
Biochemical Analysis: random blood glucose level, lipid profile (total cholesterol and triglyceride), and serum creatinine via fully automated clinical chemistry analyzer Cobas® c311 analyzer, Hitachi, Tokyo, Japan. Estimated glomerular filtration rate (eGFR) was calculated from creatinine values using standard pediatric equations.
TNF-α and Serum Fasting Insulin measurement: were quantified using a high-sensitivity ELISA kit according to the manufacturer's instructions (Quantikine, STA00C and DRG, EIA-2935, respectively).
Serum microRNA-192 Quantification: Total RNA, including small RNAs, was extracted from 200 to 400 μL of serum using RNA extraction kit optimized for biofluids (Qiagen miRNeasy Serum/Plasma Kit, Cat. No. 4427975). RNA concentration and purity were assessed using a NanoDrop spectrophotometer. RNA was reverse transcribed to complementary DNA (cDNA) using a specific reverse transcription kit for miRNAs (TaqMan™ MicroRNA Reverse Transcription Kit, Cat. No. 4366596). Mature miR-192 expression was quantified by quantitative real-time PCR (RT-qPCR) using TaqMan™ miRNA assays on a real-time PCR instrument (Applied Biosystems 7500 Fast Real-Time PCR System). Expression levels were normalized to an endogenous control miRNA (miR-16) and calculated using the ΔCt method, reported as fold change relative to controls.

2.6. Statistical analysis

Statistical analyses were performed using R version 4.3.3, with confirmation of selected analyses using MedCalc® statistical software. The distribution of continuous variables was assessed using the Shapiro–Wilk test and the Kolmogorov–Smirnov test with Lilliefors correction. All continuous variables, including age, BMI, fasting insulin, TNF-α, and microRNA-192, showed significant departures from normality; therefore, between-group comparisons were performed using the Mann–Whitney U test and continuous data were presented as median (interquartile range). Categorical variables were compared using Fisher's exact test. For multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) procedure was applied, with the FDR-adjusted q-value reported alongside the raw P-value. Bonferroni correction was additionally applied as a conservative sensitivity analysis for the between-group laboratory comparisons. Multivariable binary logistic regression was performed within the T1DM group to assess the independent associations between each biomarker and the presence of any diabetic complication. Separate models were fitted for fasting insulin, TNF-α, and microRNA-192, with adjustment for age, sex, BMI, and disease duration. Odds ratios were reported per natural unit with profile-likelihood 95% confidence intervals. Benjamini–Hochberg FDR correction was applied to the predictor P-values, and statistical significance was assessed using the corresponding q-values. A two-sided P-value <0.05 was considered statistically significant where applicable.

3. Results

The study included 200 participants: 100 children with T1DM and 100 healthy controls. The T1DM group comprised 40 males and 60 females, whereas the control group comprised 58 males and 42 females. The sex distribution differed significantly between the two groups (Fisher's exact test, $P = 0.016$).

Diabetic patients had significantly higher BMI (24.80 ± 4.30 vs. 21.38 ± 2.09 kg/m^2; $p < 0.001$) and waist circumference (73.20 ± 11.64 vs. 60.88 ± 7.62 cm; $p < 0.001$). Regular exercise was less frequent (26% vs. 58%; $p < 0.001$), and family history of diabetes was more prevalent (46% vs. 20%; $p < 0.001$). Dietary distribution differed significantly between groups ($p = 0.001$). Baseline characteristics are shown in Table 1.

3.1. Laboratory findings

The laboratory main findings were that Fasting insulin, TNF-α, and microRNA-192 were significantly altered in T1DM children. T1DM patients demonstrated significantly higher fasting insulin (10.83 ± 3.12 vs.

Table 1
Baseline demographic, anthropometric, and lifestyle characteristics of the study population.

Variable		Control ($n = 100$)	T1DM patients (n = 100)	*p*-Value
Age (years)		11.0 (9.0–12.0)	11.0 (9.0–13.0)	0.703
Sex	Male, n (%)	58 (58%)	40 (40%)	0.016
	Female, n (%)	42 (42%)	60 (60%)	
Body mass index (kg/m^2)		22.0 (20.0–23.0)	25.0 (21.5–26.3)	<0.001
Waist circumference (cm)		59.0 (55.0–66.0)	68.0 (65.0–80.5)	<0.001
Regular exercise, n (%)	Yes	58 (58%)	26 (26%)	<0.001
	No	42 (42%)	74 (74%)	
Family history of diabetes, n (%)	Yes	20 (20%)	46 (46%)	<0.001
	No	80 (80%)	54 (54%)	
Diet type, n	Low–moderate CHO	23	18	< 0.001
	Moderate–high CHO	27	49	
	High CHO	3	8	
	High protein	47	25	

Data are presented as number and percentage.
Statistical significance was defined as $p < 0.05$, p value <0.01 is highly significant.
CHO: carbohydrate
T1DM children had higher BMI and waist circumference, exercised less, had more family history of diabetes, and followed more carbohydrate-rich diets compared to healthy controls ($p < 0.001$).

3.83 ± 1.71 mIU/L; $p < 0.001$), elevated TNF-α (33.54 ± 13.17 vs. 27.73 ± 11.71 pg/mL; $p = 0.001$), and lower serum microRNA-192 expression (0.76 ± 0.34 vs. 1.27 ± 0.89; $p < 0.001$). After adjustment for multiple comparisons using the Benjamini–Hochberg false discovery rate procedure, fasting insulin, microRNA-192, and TNF-α remained significantly different between children with T1DM and controls. Fasting insulin was markedly higher and microRNA-192 was lower in the T1DM group, while TNF-α was modestly higher. TNF-α remained significant after FDR correction (q = 0.006), although the association became marginal after the more conservative Bonferroni correction (P = 0.053).

Triglycerides, total cholesterol, and random blood glucose were significantly higher in diabetic patients, reflecting metabolic dysregulation (all $p < 0.001$). eGFR was significantly lower ($p < 0.001$), which may indicate organ function changes. Laboratory findings are summarized in Table 2.

3.2. Correlation of gender with diabetic complications

The relationships between gender and the prevalence of diabetic complications; including eye, neurological, nephropathic, and cardiac complications, were assessed. The association between gender and various diabetic complications was evaluated. No statistically significant correlations were observed for eye, neurological, nephropathic, or cardiac complications, indicating that the rates of these complications were comparable between males and females (Table 3).

Patients with eye, neurological, nephropathic, and cardiac complications consistently exhibited higher BMI and fasting insulin levels compared to those without complications (all p (all p ≤ 0.05)), highlighting the role of metabolic dysregulation in disease progression. Similarly, TNF-α levels were significantly elevated in patients with all complication types ($p \leq 0.001$), indicating an underlying pro-inflammatory state. Circulating microRNA-192 levels were significantly lower in patients with eye, (p=0.049), while the association with nephropathy did not reach statistical significance ($p = 0.065$). These findings suggest that elevated BMI, hyperinsulinemia, and increased inflammatory activity are strongly linked to the presence of diabetic complications, whereas reduced microRNA-192 may reflect tissue-specific or stage-dependent molecular alterations contributing to disease pathology. These associations are presented in Table 4.

3.3. ROC curve analysis

ROC curve analysis demonstrated excellent discriminatory performance for fasting insulin, with an AUC of 0.972 (95% CI: 0.938–0.990). Using the Youden-optimal cut-off of ≥5.25 mIU/L, fasting insulin showed 97% sensitivity and 98% specificity. For microRNA-192, the classification direction was corrected because serum microRNA-192 levels were lower in children with T1DM. The corrected analysis yielded an AUC of 0.683 (95% CI: 0.613–0.747), with a Youden-optimal cut-off of ≤1.06, corresponding to 91% sensitivity and 66% specificity. TNF-α showed limited discriminatory performance, with an AUC of 0.612 (95% CI: 0.541–0.680). The Youden-optimal cut-off was >30 pg/mL, yielding 50% sensitivity and 91% specificity. ROC results are summarized in Table 5, and corresponding ROC curves are illustrated in Fig. 1.

Table 3
Correlation between gender and diabetic complications.

Complication	Gender	Present, n (%)	Absent, n	*P*-value
Eye complications	Male	8 (42.1%)	32	1.000
	Female	11 (57.9%)	49	
Neurological complications	Male	8 (42.1%)	32	1.000
	Female	11 (57.9%)	49	
Diabetic nephropathy	Male	5 (50.0%)	35	0.515
	Female	5 (50.0%)	55	
Cardiac complications	Male	8 (42.1%)	32	1.000
	Female	11 (57.9%)	49	

Fisher's exact test; no complication differed by sex. Percentages are of affected patients.

Table 2
Comparison of laboratory parameters between diabetic patients and controls.

Parameter	Control ($n = 100$)	T1DM (n = 100)	P raw	P FDR	P Bonf.
Fasting insulin (mIU/L)	3.60 (3.00–4.10)	11.0 (8.2–13.0)	7.4×10^{-31}	3.3×10^{-30}	<0.001
MicroRNA-192 (fold change)	1.07 (0.54–1.74)	0.74 (0.56–0.88)	7.9×10^{-6}	1.4×10^{-5}	<0.001
TNF-α (pg/mL)	28.0 (23.0–30.0)	31.0 (23.0–45.0)	0.006	0.006	0.053
Triglycerides (mg/dL)	148.5 (134.8–156.0)	190 (170–210)	6.2×10^{-16}	1.4×10^{-15}	<0.001
Total cholesterol (mg/dL)	156 (153–175)	189 (180–213)	1.3×10^{-19}	4.0×10^{-19}	<0.001
Random blood glucose (mg/dL)	96 (89–100)	170 (150–190)	4.6×10^{-34}	4.2×10^{-33}	<0.001
Creatinine (mg/dL)	1.00 (0.80–1.10)	0.90 (0.50–1.10)	6.2×10^{-4}	9.4×10^{-4}	0.006
eGFR (ml/min/1.73m$^{2)}$	85 (80–90)	82 (77–90)	0.002	0.003	0.020
WBCs ($\times10^3$/μL)	6.55 (4.40–8.93)	8.00 (6.70–9.10)	0.004	0.005	0.036

Median (IQR); Mann–Whitney U. FDR = Benjamini–Hochberg (primary); Bonferroni (sensitivity).
Statistical significance was defined as $p < 0.05$, p value <0.01 is highly significant.
Fasting insulin,and microRNA-192 were significantly altered in T1DM children. Triglycerides, total cholesterol, random blood glucose, and WBCs were significantly higher in diabetic patients. eGFR was significantly lower in diabetic group.

Table 4
Association between clinical biomarkers and diabetic complications.

Variable	Eye complication Present med (IQR)	Eye complication Absent med (IQR)	P FDR	Neurological complication Present med (IQR)	Neurological complication Absent med (IQR)	P FDR	NephropathyPresent med (IQR)	Nephropathy Absent med (IQR)	P FDR	Present med (IQR)	Cardiac complication Absent med (IQR)	P FDR
BMI (kg/m^2)	30.00 (25.50–30.00)	23.00 (20.00-26.00)	0.003	30.00 (26.00–30.00)	23.00 (20.00-26.00)	<0.001	30.00 (30.00–30.00)	25.00 (20.00-26.00)	<0.001	30.00 (25.50–30.00)	23.00 (20.00-26.00)	0.001
Fasting insulin (mIU/L)	15.00 (13.50–15.00)	10.00 (8.00–12.00)	<0.001	15.00 (14.00–15.00)	10.00 (8.00–12.00)	<0.001	15.00 (13.25–15.00)	11.00 (8.00–12.00)	<0.001	15.00 (13.50–15.00)	10.00 (8.00–12.00)	<0.001
MicroRNA-192	0.59 (0.49–0.83)	0.76 (0.64–0.89)	0.049	0.59 (0.49–0.83)	0.76 (0.64–0.89)	0.068	0.61 (0.48–0.84)	0.75 (0.57–0.89)	0.232	0.59 (0.49–0.89)	0.76 (0.64–0.87)	0.105
TNF-α (pg/mL)	45.00 (28.50–55.00)	29.00 (23.00–37.00)	0.019	45.00 (29.50–55.00)	29.00 (23.00–37.00)	0.014	50.00 (45.00–55.00)	29.00 (23.00–40.00)	0.027	45.00 (28.50–55.00)	29.00 (23.00–37.00)	0.017

Mann–Whitney U; Benjamini–Hochberg FDR across the 16 comparisons.
Patients with eye, neurological, nephropathic, and cardiac complications consistently exhibited high BMI, fasting insulin levels, TNF-α. In contrast, circulating microRNA-192 levels were lower in patients with eye, neurological, and cardiac complications ($p \leq 0.027$), while the association with nephropathy did not reach statistical significance ($p = 0.065$).

Multivariable analysis of biomarkers and diabetic complications: Multivariable binary logistic regression was performed to determine whether the associations between the investigated biomarkers and diabetic complications remained independent after adjustment for age, sex, BMI, and disease duration. Each biomarker was evaluated in a separate model to reduce the risk of over-fitting given the number of complication events.Fasting insulin remained independently associated with the presence of any diabetic complication (adjusted OR 2.38, 95% CI: 1.57–4.23; q = 0.003, p<0.001). In contrast, TNF-α was not independently associated with complications after adjustment (adjusted OR 1.03, 95% CI: 0.99–1.08; q = 0.202, p=0.161), while the association between microRNA-192 and complications did not reach the predefined significance threshold (adjusted OR 0.08, 95% CI: 0.01–0.83; q = 0.064, p=0.051). Disease duration remained independently associated with the presence of complications across the models. Each biomarker was fitted in a separate model adjusted for age, sex, BMI, and disease duration. Outcome = any complication (21/100 events). ORs are reported per natural unit with profile-likelihood 95% CIs. q-values represent Benjamini–Hochberg FDR-adjusted P-values.

4. Discussion

The present study evaluated tumor necrosis factor-alpha (TNF-α), fasting insulin and microRNA-192 as biomarkers of disease severity and diabetic complications in Egyptian children with Type 1Diabetes Mellitus (T1DM). Changes in circulating microRNA profiles have been documented in the pathogenesis of T1DM and are linked to immune and inflammatory signaling pathways involved in disease related complications, highlighting their value as mechanistic biomarkers.[17] The interaction between microRNAs and inflammatory signaling pathways is increasingly acknowledged as a factor in the development of diabetic complications, with aberrant miRNA expression connecting metabolic stress to immune activation.[18] Elevated levels of pro-inflammatory cytokines, including TNF-α, have been observed in individuals with T1DM and are linked to immunometabolic dysfunction.[19] It was also stated that reduced insulin sensitivity, indicative of insulin resistance, in patients with type 1 diabetes is associated with an increased prevalence of microvascular complications, including neuropathy, retinopathy, and nephropathy.[20]

The findings demonstrate that fasting insulin is a strong discriminator between diabetic patients and healthy controls and is consistently associated with multiple diabetic complications. In contrast, TNF-α and microRNA-192 showed moderate but biologically meaningful associations, suggesting complementary roles in the inflammatory and epigenetic mechanisms underlying disease progression.

A key finding of this study is the markedly elevated fasting insulin levels observed in children with T1DM compared to healthy controls, with highly significant statistical differences. Moreover, fasting insulin levels were consistently higher in patients with eye, neurological, cardiac complications, and diabetic nephropathy. The strong diagnostic performance of fasting insulin, reflected by an excellent area under the ROC curve, indicates its potential utility as a biomarker of disease burden rather than merely disease presence.

Although T1DM is classically characterized by insulin deficiency, elevated fasting insulin levels in this cohort may reflect compensatory hyperinsulinemia secondary to insulin resistance, increased adiposity, or intensive insulin therapy. The significantly higher BMI and waist circumference observed in diabetic children support the presence of metabolic stress, which may contribute to increased insulin requirements and promote inflammatory and vascular injury. These findings suggest that fasting insulin may serve as an integrated marker of metabolic dysregulation and complication risk in pediatric T1DM.

Earlier studies have shown that T1DM patients with elevated fasting insulin levels or reduced insulin sensitivity are at greater risk for microvascular complications, including neuropathy and retinopathy.[20,21] These observations are in line with our findings, suggesting

Table 5
ROC analysis of investigated biomarkers

Marker	AUC(95% CI)	Direction	Cutoff	Sensitivity (%)	Specificity (%)	
Fasting insulin	0.972(0.938–0.990)	Higher	≥ 5.25	97	98	<0.001
MicroRNA-192	0.683 (0.613–0.747)	Lower	≤ 1.06	91	66	<0.001
TNF-α	0.612 (0.541–0.680)	Higher	>30	50	91	0.008

MedCalc; 95% CI; Youden-optimal cut-off. Fasting insulin demonstrated excellent discriminatory ability, with an area under the curve (AUC) of 0.972. At the optimal cut-off of 5.25 mU/L, fasting insulin achieved 97% sensitivity and 98% specificity. In contrast, microRNA-192, while highly sensitive (91%), exhibited low specificity (66% and an AUC of 0.683. TNF-α showed sensitivity of (50%), specificity of 91%) and an AUC of 0.612.

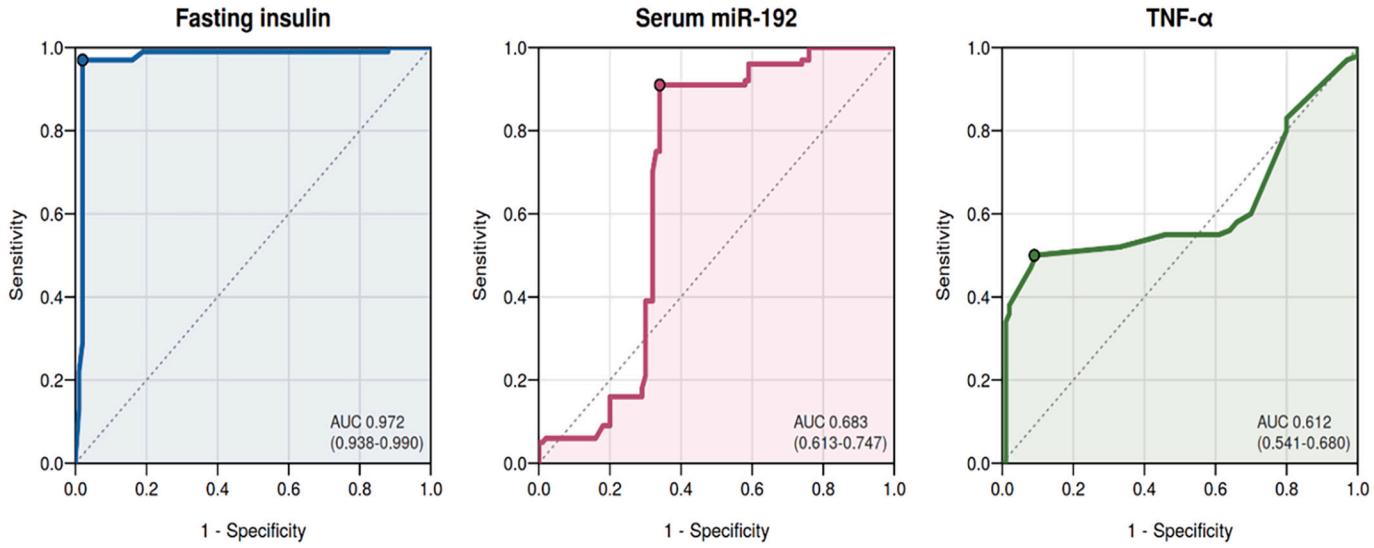


Fig. 1. ROC curve for fasting insulin, micro RNA 192 and TNF.
ROC curves for discrimination of T1DM from controls, with T1DM as the positive class. Filled circles mark the Youden-optimal operating points.

that hyperinsulinemia may indicate underlying metabolic stress and early vascular injury. Importantly, whereas most previous research focused on adults or mixed-age groups, our study specifically examines pediatric patients, highlighting the value of detecting biomarkers at an early stage.

TNF-α levels were significantly elevated in children with T1DM compared to controls and were further increased in patients with eye, neurological, and cardiac complications. This supports the role of chronic low-grade inflammation in the pathogenesis of diabetic complications, even at a young age. TNF-α is known to impair insulin signaling, promote endothelial dysfunction, and contribute to vascular injury, which may explain its association with neurovascular and cardiac complications in this study.[22]

Our findings are consistent with previous reports demonstrating elevated TNF-α levels in T1DM and their association with the severity of disease complications. Several studies have reported particularly strong links with cardiovascular and neurovascular complications, which parallels our observations.[23]

MicroRNAs (miRNAs) are short non-coding RNA molecules that regulate gene expression at the post-transcriptional level and are increasingly recognized as key players in the pathogenesis of diabetes and its complications. The dysregulation of miRNA expression can influence multiple pathways involved in complications such as nephropathy, retinopathy, neuropathy, and cardiovascular disease. Micro RNA-192 has been shown to modulate key signaling pathways, including TGF-β, SMAD, and PTEN/PI3K/AKT, thereby influencing extracellular matrix accumulation and fibrotic processes that drive the progression of renal damage. Although certain clinical investigations suggest that reduced circulating miR-192 levels may serve as an early indicator of nephropathy, experimental studies demonstrate that upregulation of miR-192 within renal tissue can enhance fibrogenesis and accelerate disease progression.[13] Similarly, miRNAs such as miR-155 and miR-146a have been associated with inflammatory and endothelial pathways implicated in retinopathy and other microvascular complications. Collectively, these findings suggest that miRNAs may function not only as potential biomarkers but also as molecular mediators linking metabolic imbalance to tissue damage in diabetes-related complications.[24]

Persistent hyperglycemia promotes oxidative stress and endothelial dysfunction mechanisms in which microRNA-192 associated with.[25] Consequently, reduced expression of this miRNA may facilitate amplified inflammatory responses and vascular injury within these tissues. In the present study, circulating microRNA-192 levels were significantly lower in patients with eye, compared to those without complications, although microRNA-192 levels were lower in patients with neuropathy, nephropathy, cardiac complications, the difference did not reach statistical significance.

Although individuals with nephropathy exhibited lower levels of microRNA-192, the association did not achieve statistical significance. Previous studies have described a complex and occasionally conflicting role for microRNA-192 in diabetic nephropathy.[13,26] The absence of statistical significance in our cohort implies that the association between circulating microRNA-192 and nephropathy could be stage-specific or modulated by additional contributing factors.

In this study, BMI was calculated and evaluated as an associated factor with diabetic complications, including retinopathy, neuropathy, nephropathy, and cardiac involvement. Patients with complications across all categories demonstrated significantly greater BMI values compared to those without complications. These findings support the concept that excess adiposity contributes to the progression of both microvascular and macrovascular damage in diabetes. Increased BMI is known to increase insulin resistance, promote chronic low-grade inflammation, and enhance oxidative stress, all of which accelerate

endothelial dysfunction and tissue injury. Recent evidence continues to highlight obesity as an independent risk factor for diabetic nephropathy progression and cardiovascular disease, even after adjustment for glycemic control.[27,28]

Similarly, large cohort studies have reported that higher BMI is associated with increased risk of diabetic peripheral neuropathy and retinopathy, potentially through inflammatory and metabolic pathways that impair microvascular circulation.[29] The strong associations observed in our cohort reinforce the importance of weight management as part of comprehensive strategies aimed at reducing long-term complication burden in patients with diabetes.

Diabetes is a chronic condition that adversely affects multiple organs as a result of persistent high blood glucose levels and therefore requires continuous management. Evidence indicates that complications, hospitalizations, and mortality related to diabetes occur more frequently among older adults with the disease.[30,31] In this study on children statistical analysis indicates that there is no clear correlation between age and the occurrence of diabetic nephropathy, as the outcomes are evenly distributed across all age groups from 5 to 16 years, which suggests that age does not have a significant impact on the presence or absence of diabetic nephropathy in this sample.

Some studies reported the correlation of gender and diabetic complications; they found that diabetes mellitus especially type 2 is often less well controlled in women across various metabolic parameters. This may be partly because women generally have lower insulin sensitivity than men, which leads to the need for higher doses of insulin to maintain appropriate blood glucose levels and meet therapeutic targets.[32] Differences in glucose regulation between men and women are particularly noticeable in the prediabetic stage. Women tend to have lower fasting glucose levels but are more likely to develop impaired glucose tolerance after meals or following a glucose load.[33] During puberty, the incidence of Type 1 diabetes is lower in girls, partly because they tend to retain stronger residual β-cell function compared with boys. This observation suggests that female gonadal hormones may provide temporary protection against the development of Type 1 diabetes. Moreover, adolescents with lower levels of circulating estrogen appear to have a higher likelihood of developing this form of diabetes.[34,35] In our study on type 1 in children, we found no significant correlation between gender diabetic complications whether eye, neurological, nephropathic or cardiac complications. Importantly, multivariable analysis provided a more conservative assessment of the relationship between the biomarkers and diabetic complications. After adjustment for age, sex, BMI, and disease duration, fasting insulin remained independently associated with the presence of any complication, whereas the associations of TNF-α and microRNA-192 were no longer statistically significant after FDR correction. These findings indicate that the crude associations observed for TNF-α and microRNA-192 may be partly explained by clinical factors, particularly disease duration. Thus, the present findings should not be interpreted as evidence that TNF-α or microRNA-192 independently predicts diabetic complications.

In the present study, fasting insulin demonstrated excellent statistical discrimination between children with T1DM and controls, with an AUC of 0.972. However, this finding should be interpreted cautiously because all participants with T1DM were receiving exogenous insulin therapy. Conventional insulin immunoassays measure total circulating immunoreactive insulin and cannot distinguish endogenous insulin from administered insulin. Although blood sampling was performed before the morning rapid-acting insulin dose, residual basal insulin may still have contributed to the measured concentrations. Therefore, the observed elevation in fasting insulin should not be interpreted as a direct measure of endogenous β-cell reserve. Rather, it may reflect overall insulin exposure and metabolic burden, potentially related to insulin resistance and the coexistence of obesity or other features of double diabetes. Larger studies incorporating detailed insulin dosing, insulin resistance measures, and appropriate assessment of endogenous insulin secretion are required to clarify the clinical utility of fasting insulin in pediatric T1DM. Elevated fasting insulin levels may reflect insulin resistance, metabolic dysregulation, or compensatory pancreatic activity, all of which are known to contribute to vascular dysfunction, inflammatory activation, and progression of diabetic complications.[36] Importantly, the identification of a clinically relevant cut-off value (5.25 mU/L) enhances the translational value of our findings. This threshold may serve as a practical decision-making point in clinical settings, although validation in larger and independent cohorts is necessary before routine implementation. Overall, the ROC analysis indicates that fasting insulin is a highly accurate biomarker in this population, with near-perfect discrimination capacity. These findings highlight its potential role in early detection, risk stratification, and possibly monitoring disease progression in patients at risk of complications.

The ROC analysis for microRNA-192 yielded an AUC of 0.683, with 91% sensitivity and 66% specificity at the Youden-optimal cut-off. This indicates modest discriminatory ability rather than near-perfect diagnostic performance. The corrected direction of classification is consistent with the lower serum microRNA-192 levels observed in children with T1DM. These findings suggest that microRNA-192 may have potential as part of a broader biomarker panel, but its performance as a standalone diagnostic marker appears limited and requires validation in independent pediatric cohorts. When comparing our ROC results for miRNA-192 with previous literature, notable differences emerge. For example, in studies of diabetic kidney disease (DKD), miRNA-192 has often demonstrated moderate to good diagnostic performance. In a case-control study of type 2 diabetic patients, miRNA-192 showed an AUC of 0.79 with 65% sensitivity and 92.5% specificity, indicating reasonable discriminatory ability in distinguishing patients with DKD from healthy controls.[37]

Furthermore, another study suggests that circulating miRNAs, including miR-192, can serve as promising non-invasive biomarkers for early DKD diagnosis, with pooled estimates for sensitivity and specificity around 0.76 and 0.74, and an overall AUC near 0.79.[38] This discrepancy underscores that the performance of miRNA 192 appears to vary substantially depending on disease type, clinical setting and maybe the population.

Additionally, several studies in DKD have reported that miRNA-192 expression and diagnostic accuracy can differ based on the biological source (serum vs. urine) and disease progression stage. In one investigation, both serum and urine miRNA-192 levels were found to change with advancing DKD, and combined serum-urine detection improved specificity and reduced misclassification compared to single sample types.[39]

TNF-α demonstrated limited discriminatory performance in the present study, with an AUC of 0.612. Although the Youden-optimal cut-off showed relatively high specificity (91%), sensitivity was only 50%, indicating that TNF-α alone is unlikely to provide adequate diagnostic discrimination. This supports the interpretation that inflammatory biomarkers such as TNF-α may be more informative when combined with other metabolic or disease-specific markers rather than used as standalone diagnostic tests.

The diagnostic performance of TNF observed in our study aligns with reports that TNF alone often has limited discriminatory ability as a single biomarker as in this study on tuberculous pleural effusion, its sensitivity and specificity is ~0.85 and ~ 0.80, with an overall AUC of 0.89, suggesting reasonable but not definitive diagnostic power.[40] Combining TNF with other inflammatory cytokines or disease-specific biomarkers such as IL-6 and IFN-γ has been shown to enhance sensitivity and specificity, supporting the approach of multi-marker panels over single-marker diagnostics.[41]

This study is a well-characterized pediatric cohort with age matched controls, comprehensive assessment of metabolic and inflammatory biomarkers, and evaluation of multiple diabetic complications and ROC assessment. However, it has few limitations first, its cross-sectional design prevents determining causal or temporal relationships between biomarkers and diabetic complications. Second, missing data on

pubertal status and partial clinical remission introduces potential residual confounding, as puberty directly impacts insulin sensitivity and requirements. Third, because all participants with T1DM were on exogenous insulin, conventional immunoassays could not differentiate it from endogenous insulin; thus, fasting insulin levels may include residual basal insulin and should not be viewed as a direct measure of β-cell reserve. Finally, the small sample size, single-center design, and lack of detailed complication grading may limit the generalizability and precision of our findings. Larger, multicenter prospective studies with detailed clinical tracking are needed to validate these results.

5. Conclusion

In conclusion, this study demonstrated significant differences in fasting insulin, TNF-α, and microRNA-192 between children with T1DM and healthy controls. ROC analysis showed excellent statistical discrimination for fasting insulin, whereas microRNA-192 demonstrated modest discriminatory ability and TNF-α showed limited discriminatory performance. However, interpretation of fasting insulin as a biomarker is limited by exogenous insulin therapy, as conventional immunoassays cannot distinguish endogenous from administered insulin. In multivariable analysis, fasting insulin remained independently associated with the presence of diabetic complications after adjustment for age, sex, BMI, and disease duration, whereas TNF-α and microRNA-192 did not retain independent statistical significance after FDR correction. These findings support the potential relevance of metabolic factors in pediatric T1DM complications but should be considered exploratory. Larger prospective studies with detailed assessment of insulin therapy, pubertal status, glycemic control, and longitudinal outcomes are required to determine the clinical utility and predictive value of these biomarkers.

CRediT authorship contribution statement

Amal Ahmed Mohamed: Writing – original draft, Validation, Supervision, Project administration, Methodology. **Noha Mohamed Mesbah:** Writing – review & editing. **Asmaa Ramadan Abdel-Hamed:** Writing – original draft. **Kawkab Mtwaly:** Writing – original draft. **Eman El Sayed Mohamed Habib:** Validation, Supervision, Investigation. **May Mohsen Samy Selim:** Methodology. **Salma Mohamed Saed:** Methodology. **Mohamed Abdelfattah Ibrahim Saad:** Visualization, Investigation. **Heba Salah Shahin:** Software, Formal analysis. **Sherihan Hassan Elkady:** Writing – review & editing, Writing – original draft, Methodology. **Amira N. Ahmed:** Writing – review & editing, Methodology. **Nourhan Mekky:** Writing – review & editing, Writing – original draft, Methodology. **Weam Shaheen:** Software, Data curation. **Hoda H. Ahmed:** Resources, Investigation. **Tarek Abdelkader Sallam:** Software, Formal analysis. **Nehal M. Khairy:** Software, Methodology. **Eman Alsayed Abouahmad:** Supervision, Methodology, Conceptualization.

Consent to publish declaration

Not applicable.

Clinical trial number

Not applicable.

Human ethics approval declaration

National Nutrition Institute's Ethics Committee (approval number IN000154) in accordance with the principles of the Declaration of Helsinki for human research.

Consent to participate declaration

Consent was taken in an Arabic form under regulations of National Nutrition Institute.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used [Chat GPT] to [rephrase some sentences]. After using this service, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

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Declaration of competing interest

The authors do declare that they have no known competing financial or non-financial interests or personal relationships that could directly or indirectly have appeared to influence the work reported in this article.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

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