Assessment of Leptin, TNF-α and Visfatin Markers in Type 1 Diabetic Children

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Abstract:

Objectives: The main objective of this research is to provide statistical predictive model(s) to identify patients with high-risk ""*type 1 diabetes mellitus*"". Furthermore, we aimed to evaluate visfatin, leptin, and TNF-α in children with T1DM regarding glycemic state and their BMI and use these statistical analytics solutions with the logistic regression predictive model results to check the diagnostic potential for these markers in the future treatment of T1DM.

Methods: The study included a hundred diabetic patients, which were classified based on HbA1c level into controlled and uncontrolled groups. They were also categorized according to their BMI: normal weight, overweight, and obese. ELISA and real-time PCR were used for detection.

Results: There was an elevation in leptin and TNF-α serum, while C-peptide and visfatin decreased significantly in patients.The uncontrolled diabetics showed higher leptin and TNF-α but lower C-peptide and visfatin than controlled diabetics. While increasing leptin gene expression and TNF-α levels in uncontrolled diabetic group but visfatin decreases.

Conclusion: The uncontrolled diabetic group was associated with higher weight, BMI, and higher leptin gene expression than controlled diabetics. We found a significant impact of both markers on the prediction of T1DM with high sensitivity and specificity and even with the acceptable significance of *p*-values.

Keywords: *Type 1 Diabetes Mellitus (T1DM); Visfatin; Leptin; Obesity; BMI; HbA1c*

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1. *Introduction*

Type 1 diabetes mellitus (T1DM) is a prevalent autoimmune disorder among children (5–10% of cases). It leads to the death of pancreatic β-cells, causing insulin deficiency. Genetic susceptibility, in synergy with environmental triggers, contributes to the emergence of an immune reaction against pancreatic β-cells' self-antigens, leading to a gradual loss of β-cells and, eventually, an imbalance in glucose homeostasis [1].

Obesity has turned into a major public health issue this century, affecting adults and children [2]. The incidence of obesity among adolescents has increased drastically in recent decades, and it has been identified as the main issue confronting youth today [3]. Surprisingly, obesity and overweight are increasingly common among T1D children, and recent research found that T1D youth tend to be more obese than others without the disease. Excess fat buildup typically results in various metabolic problems, leading to a substantial reduction in life quality[4].

Recognition of adipokines associated with diabetes might provide new chances for clinicians for early diagnosis and better control of diabetes, obesity, and their associated complications. In diabetesand metabolic disorders, metabolic hormones (such as resistin, leptin, and visfatin) and pro-inflammatory cytokines (such as TNF-α) were also altered [5].

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory adipokine that is secreted in large amounts in adipose tissue. It plays a critical role in inflammation and autoimmune diseases, correlates with obesity, and decreases with weight loss [6]. TNF-α assists the up-regulation of microalbuminuria forT1DM patients[7]

Many adipokines have been linked to diabetes mellitus and play a role in multiple metabolic pathways such as regulation of adipogenesis, energy expenditure, insulin sensitivity, and appetite control as leptin, visfatin, omentin-1, and cardiotrophin-1 [8].

Omentin-1 is secreted from the visceral fat adipose tissue with insulin-sensitizing effects and more dominant in diabetes mellitus prognosis.

Different studies found an association between concentrations of omentin-1 and diabetes mellitus, even increasing or decreasing concentrations associated with the disease[9].

Cardiotrophin-1 (CT-1) is a protein that was isolated from the supernatant of mouse embryonic corpuscles. It is highly expressed in the heart, skeletal muscle, liver, lung, and kidney. Lower levels of CT-1 expression are also seen in testis, brain, and adipose tissue[10].CT-1 has a glucose-lowering property or a role in glucose homeostasis, and this adipocytokine has the ability to promote insulin-stimulated glucose uptake in adipocytes [11].

Visfatinis an adipokin produced by visceral adipose tissue, from which it acquired its name "visfatin," as it is produced in high amounts in visceral fat. It is an autocrine, paracrine, and endocrine peptide that is multifunctional; it contributes to the biosynthesis of nicotinamide, enhancement of cell proliferation, and hypoglycemic effect [12]. Insulin sensitivity in the liver is regulated by the autocrine effects of visfatin. This hormone is found in the nucleus and the cytoplasm of cells and has been identified in some organs and tissues such as the brain, lungs, testis, spleen, kidney, and testicles [13].

Leptin, secreted by adipocytes, is a 16-kDa protein hormone. Its concentration is directly proportional to the mass of the body fat. Also, it regulates energy expenditure and food intake, maintaining the stores of body fats [14]. It has been proposed that leptin has a link between obesity, cardiovascular risk, and diabetes. Serum leptin regulates blood glucose through two different brain pathways: controlling appetite and stimulating liver-glucose functions [15].

The goal of the current research is to investigate the capabilities of statistical predictive model(s), namely, logistic regression as a new data-driven solution in identifying the patients with T1DM diabetes and the future prediction of treatments based on the use of numerous of predictors that were given in the data in-hand. Next, we compare the levels of **leptin, visfatin,** and ***TNF-α*** in controlled and uncontrolled ***obese insulin-dependent diabetes mellitus children (T1DM)***. In addition to evaluating leptin gene expression in diabetic children according to BMI and glycemic control. Also, we aimed to assess the value of studied markers as predictors of T1DM and uncontrolled diabetic patients. The comparative studies and results are tabulated below. The obtained results were investigated under the following accuracy of statistical quality of measures: Both ordinary least-squares regression and logistic regression model(s), the fitness of predictions based on both correlations R-squared and root mean=squared errors, and the significance of *p*-values, Negelkerke Pseudo-R2, Goodness of fit through the comparison between models (One utilizing the predictors' input data, and the model that is not utilizing any of the given predictors' input data. We note that the likelihood ratio test (G2) does not always perform well, especially when data are sparse. We tabulated most of these quality measures, including the classification accuracy based on the correct classification rate (CCR); which is part of the confusion matrix including Sensitivity, Specificity, Goodness of Fit (G) or Chi-Squared values; F-statistics, positive likelihood ratio (PLR), Negative likelihood ratio (NLR), Positive Predictive Value (PPV), and Negative Predictive Value (NPV), false-positive rate (FPR), and false-negative rate (FNR), Diagnostic odds ratio (DOR), and accuracy of both Type-I and Type-II errors.

2. *Data acquisition, Subjects, and Research Methods:*

This study was carried out using the most common strategy of gathering data from a group of patients. We carried out the state-of-the-art statistical analysis with deep predictive models for visualization and explanatory analysis of our obtained results and drew the proper conclusions.

The gathered data were one hundred children (59 males and 41 females, with an age of 9.6 ±3.1 years, ranging from 6 to 12 years). They were diagnosed with T1DM, fitting the criteria of the International Society for Pediatric and Adolescent Diabetes (ISPAD) (16). The

ISPAD criteria include the following measures: Fasting blood glucose > 126 mg/dl, Hba1c >6 %, and C-peptide levels <1 ng/ml. Regular follow-ups were performed in the outpatient clinic of the endocrinology unit in Mansoura University Children's Hospital. The presence of patients with significant medical disorders or use of medications rather than insulin, heart failure, hepatic or renal diseases, and myocardial infractions were excluded from this study.

This prospective study also included a control group of 50 healthy normoglycemic children of ages and genders with no significant difference from the other group, as it is shown in the obtained tabulated results in Tables II through IV.The Ethical Committee of the Faculty of Medicine, Zagazig University, Egypt, authorized this work. The demographic data of the studied individuals are summarized in **(Table 1).** All children's parents who participated in this study had signed written consent before the beginning of the study. To the Center for Disease Control and Prevention, patients were categorized into the following criterion:

Three different categorical groups: {**Group IIa** withn=39} 39% "normal weight" in which (23% are controlled diabetic and 16% are uncontrolled diabetic), {**Group IIb**with n=32} 32% "overweight" in which 10% are controlled and 22% are uncontrolled diabetic, and {**Group IIc**withn=29} 29% "obese". (in which 12% are controlled diabetic and 17% are uncontrolled diabetic) based on BMI plotted standard by age on the sex or gender-specific growth chart [17].

- An additional classification was added to patients based on HbA1c level [18], classifying the patient group according to the literature and best practice of the national benchmarks into the following: controlled diabetic group (HbA1c ≤7.0 %,**Group Ia**n=46) and uncontrolled diabetic group (HbA1c >7.0%, **Group Ib**n=54).

The following laboratory tests were performed on all subjects:

2.1 *Biochemical investigations*

Determination of glucose, HbA1c, lipid profile, TNF–α, C-peptide, visfatin, and leptin serum level was performed after overnight fasting for 8-10 hours. Fasting blood glucose levels were determined enzymatically using Spinreact diagnostic kits (San Antonio, Claret, Texas, USA). Glycosylated hemoglobin (HbAlc) was measured with a nephelometric technique (MISPA i2, AGAPPE Diagnostics GmbH, Switzerland).Serum triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were measured with enzymatic colorimetric endpoint methods using kits from (Spinreact, Spain). Low-density lipoprotein (LDL-C) was estimated by applying the Friedwald Equation. Quantification of C-peptide was performed on an immulite 2000 analyzer, Germany.

The levels of serum leptin, visfatin, and TNF–α were measured by commercially available enzyme-linked immunosorbent kits from (RayBiotech, Inc., Georgia, USA) for visfatin

(Immunospec, 7018 Canoga Park, CA 91303, USA) for leptin and (Avibion, Helsinki, Finland) for TNF-α.

2.2 *Gene expression of leptin by Real-time PCR*

Extraction of total RNA was accomplished using the RNeasy Mini kit (QIAGEN GmbH, Germany), following the manufacturer's instructions. We performed additional DNase digestion during the extraction processes. The sample was treated with DNase for two hours at 37°C using 10 U/µL of DNase I and 20 U/µL of RNase inhibitor (Invitrogen Ltd., Paisley, UK) every 5 µg of RNA. We analyzed the RNA concentration at an absorbance of 260 nm using a spectrophotometer instrument (Nanodrop 2000, Thermo Scientific, USA). The purity of the sample was determined based on the ratio of absorbance at 260 nm to that at 280 nm and then visualized by 1.5% agarose gel electrophoresis to detect the two ribosomal distinct bands. 1 µg of total RNA was converted to cDNA using the RT2First Strand Kit (QIAGEN Science, Maryland, USA).

We used Real-time PCR analysis (Rotor-Gene Q, Qiagen) to detect gene expression. We amplified 3µl (about 30M) of the cDNA using 10pmol of each primer pair, 10µl of 2× RT2SYBR Green Master Mix (QIAGEN Science, Maryland, USA), and the final volume was completed to 20µl using nuclease-free water. The cycling parameters of the PCR amplification were initial denaturation at 95 °C for 3 min, then 40 cycles of amplification (denaturation at 94°C for 20 seconds, annealing at 58°C for 30 seconds, and extension at 60°C for 30 seconds). The primer design was performed online at the NCBI site. The primer sequences for Leptin are GACTTCATTCCTGGGCTCCA (sense) and GGATCACGTTTCTGGAAGGC (antisense).

In comparison, the sequences of the GAPDH primers were TCCATGACAACTTTGGCATCGTGG(sense)andGTTGCTGTTGAAGTCACAGGAG AC (antisense). For the relative quantification of target genes, we used a mathematical model proposed by Pfaffl[19]. The fluorescence produced by the fluorochrome upon binding of SYBR Green to double-stranded DNA was used to quantify gene expression. In the dissociation curve, a single peak per amplification product was regarded to show purity. For the relative measurement of gene expression, we utilized the comparative Ct technique (ΔΔCt) in this research, and we compared the gene expression of patients to that of the healthy group. As an internal control and for normalization, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was utilized.

2.3 *Statistical analysis*

We used the statistical package for social sciences (SPSS) software version 21 for analyzing the data. Continuous variables were expressed as mean and standard deviation (SD). The difference between the means of two data sets was assessed using the student's t-test. We utilized the chi-square test to identify the association between categorical variables. *P* values < 0.05 were considered statistically significant. We created the receiver operating characteristic (ROC) curve to determine the optimal cut-off of the investigated markers (leptin and visfatin) and to differentiate between patients and control subjects, as well as between controlled and uncontrolled patients. Finally, univariate and multivariate linear regression models were estimated to determine important predictive variables of diabetes.

3. Results:

The patient group showed higher weight ($p < 0.001$) and a similar height in comparison with the healthy group. The BMI in patients with T1DM was significantly higher ($p < 0.001$) than in the healthy group. Patients were divided according to BMI into **obese** (N=29, 29% with Z-score= 2±0.4) and percentiles ≥ 95%), **overweight** (N = 32, 32% with Z-score= 1.2±0.2 and 85% < percentiles <95%), and **healthy weight** (N = 39, 39% with Z-score = 0.6±0.2 and 5% <percentiles <85%).

Laboratory and demographic data are presented in **(Table I)**. It showed a significant elevation in glucose, HbA1c, total cholesterol, triglyceride, LDL, TNF-α, and leptin serum levels and a significant decrease in HDL, C- peptide, and visfatin serum levels in patients in comparison with the healthy group ($p < 0.001$).

Non-Diabetic		*Diabetic*						
		HbA1c Based			BMI Based			
	Control	HbA1c Controlled	HbA1c uncontrolled		Normal BMI	Overweight BMI	Obese BMI	
Group Index	N	Ia	Ib		IIa	IIb	IIc	
Cases	50	46	54	*p*-value 1	39	32	29	*p*-value 2
Age	9.5±2.5	9.4±2.5	9.7±3.1		9±2.1	9.6±2.7	10.3±2.3	
Height cm	143.9±8.5	146.7±8.4	149.3±11		148.9±9	148.7±8.9	142±9.7	
Weight kgm	40±8.2	43.2±7.4	48±11.3		41.6±9.3	47.3±9.7	46.2±8.8	
BMI	20.9±2.5	20.01±2.5	21.2±3.2	0.092	18.5±2.1	21.1±2.5	22.6±2.2	<0.001
HBA1c%	4.4±0.8	6.7±0.34	9.1±1.3	<0.001 T	7.7±1.4	7.9±1.7	8.5±1.4	0.084
Glucose mg/dl	89±11	196±27	214±32	0.005	197±23	207±31	216±38	0.137
TG mg/dl	70±17	85±23	117±39	<0.001 T	99±28	105±29	103±25	0.081
Cholesterol mg/dl	120±18	147±25	178±40	<0.001 T	155±36	165±40	174±32	0.070
HDL-C mg/dl	51±1	47±5	43±6	0.002	46±6	44±7	44±5	0.121
LDL-C mg/dl	55±15	83±26	110±24	0.001	89±29	99±31	106±27	0.114
TNFα pg/ml	11.1±3.4	19.3±3.8	21.1±3.3	0.042	19.8±3.6	19±3	22.4±3.3	0.001
C- peptide ng/ml	3.8±0.8	0.7±0.2	0.3±0.1	<0.001 T	0.6±0.2	0.6±0.1	0.4±0.1	0.105
Visfatinng/ml	28.7±4.4	11.9±3.9	10.8±3	0.248	13.5±3.9	11.1±3.4	8.5±2.5	<0.001 T
Leptinng/ml	4.2±1.2	10±3.3	12.9±3.8	<0.001 T	7.4±1.7	12±0.9	16.8±3.3	<0.001 T

(Table I) Demographics and laboratory data for the healthy control group and diabetic children. Classification based on either HbA1c level or BMI category

SD, standard deviation; T, independent t-test.*P*-value 1 < 0.05 indicates a significant difference

between controlled and uncontrolled Hba1c-based patient groups. *P*-value 2 < 0.05 indicates a significant difference between BMI-based patient groups.

Concerning the BMI, no significant differences were demonstrated in the outcome data between obese, and healthy groups. However, we found a significant increase ($p < 0.001$) in TNF-α, and leptin serum levels and a significant decrease ($p < 0.001$) in visfatin serum level with BMI.

Controlled T1DM patients (with Z-score= 1.09±0.6) exhibited significantly decreased levels of HbA1c in comparison with the uncontrolled group. However, no significant differences concerning sex and age between these groups were found.

The uncontrolled diabetic patients (with Z-score= 1.3±0.7) were associated with higher weight and BMI ($p = 0.043$ and 0.032, respectively).

Uncontrolled diabetic patients were significantly associated with higher glucose, HbA1C, TG (triglyceride), TC (total cholesterol), LDL, TNF-α, leptin, and lower HDL, and C peptides compared to controlled diabetic patients.

(Table II)showed considerable AUCs for leptin and visfatin for distinguishing between patients and control groups (AUC = 0.976 and 0.997, respectively). Therefore, leptin and visfatin can be predictive factors for type 1 diabetes**(Figure 1)**. The cut-off values and performance characteristics of the (ROC) curve for discrimination between controlled and uncontrolled diabetic cases showed poor AUCs for leptin and visfatin (AUC = 0.668, 0.604,

		AUC	Cut off	Sensitivity	Specificity	PPV	NPV	PLR	NLR	Accuracy	*p*
Healthy To patients	**Visfatin**	0.997	<19.6	97	`100	100	94	∞	0.03	98	0.857
	95%CI	0.992-1		94-98	100-100	100-100	93.7-97.7	0	0.01-0.4	97.3-99	
	Leptin	0.976	6.35	87	94	97	78	14.5	0.14	89	-
	95%CI	0.958-0.994		86-94	91.5-95.6	84.7-100	76.3-93.2	10.9-17.6	0.11-17	87.5-91.5	
Controlled diabetic to Uncontrolled	**Visfatin**	0.604	<13.59	72	41	59	56	1.22	0.68	58	0.857
	95%CI	0.493-0.715		71.2-78.3	39.4-42.2	57.8-62.1	55-58	1.1-1.5	0.61-72	56.6-59.7	
	Leptin	0.668	12.95	46	76	69	55	1.92	0.71	60	
	95%CI	0.563-0.773		43.4-48.5	74.5-78.2	68.7-70.2	53.4-57.8	1.76-2.1	0.69-0.72	59.5-61.5	

respectively).

Table (II): AUC and performance criteria of *leptin* and *visfatinlevels* for discriminating between patients and healthy control group and between controlled and uncontrolled diabetic children.
AUC: area under ROC curve, PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio, *P*: comparison between leptin AUC versus visfatin AUC

Since our focus in this research is to determine the relationship and significant impact of both biomarkers leptin and visfatin on diabetes and levels of HbA1c; then by looking at the given data, we have seen that the correlation between the two biomarkers and HbA1c is: 0.378 and 0.413; respectively; which is not that strong. Therefore, we followed an adequate scheme to convince us to use the statistical predictive model and to be able to help in identifying the future diabetic patients. We carried the comparative studies among the means (H0: mu1 =mu2; and H1:mu1≠mu2) of individuals with normal and high HbA1c in both markers.

Results are shown that there is a significant difference with a p-value <0.001 for both leptin and visfatin. In the end, we have a great indicator to move forward and utilize the regression model, and as the results are tabulated in tables III-IV, we were able to see such a significant impact.

By increasing visfatin concentration, there is a significant increase in TC, LDL, TNFα, HbA1c, and a significant decrease in C-Peptide, and HDL, and by increasing leptin concentration, there is a significant increase in BMI percentiles **(figure 2B)**, HbA1C % **(figure 2A)**, glucose, and TNFα, and a significant decrease with C-Peptide and HDL. **(Table III).**

Table (III): Correlation between laboratory data concerning *leptin* and *visfatin levels*with other parameters in all studied T1DM children.

	Visfatin		Leptin	
	r	***p*- value**	***r***	***p*-value**
BMI	-0.440	<0.001	0.940	<0.001
Glucose	0.261	0.009	0.312	0.002
HbA1c	0.413	<0.001	0.752	<0.001
TG	0.188	0.061	0.041	0.688
Total Cholesterol (TC)	0.379	<0.001	0.250	0.012
HDL	-0.280	0.005	-0.200	0.046
LDL	0.316	0.001	0.221	0.027
TNFα	0.726	<0.001	0.359	<0.001
C- peptide	-0.286	0.004	-0.238	0.017

We assessed Gene expression relative to that of normal children **(Figure 3)**. Leptin gene was expressed in children with diabetes type 1 with a median of 4.19 (range 0.10-16.35) folds (Figure 4a), more than the normal control group with a median of 0.96 (range 0.58-1.41). According to BMI, the expression of leptin level increased significantly, reaching its maximum in obese diabetic patients with a median of 8.35 (range 0.82-16.35) folds (Figure 4b). Uncontrolled diabetic children were associated with significantly higher leptin gene expression (Figure 4c) with a median of 10.4 (2.06-16.35) folds as compared to the controlled diabetic group with a median of 2.19 (0.10- 4.91).

On the other hand, leptin gene expression showed significant positive correlations with the uncontrolled diabetic group ($p \leq 0.001$), glucose ($p \leq 0.001$), TNFα ($p \leq 0.001$), and leptin protein level ($p = 0.046$), and negative correlations with C-peptide ($p < 0.001$) and visfatin ($p < 0.005$).

We implemented logistic regression analysis to predict uncontrolled diabetes within all studied cases, using age, gender, obesity, laboratory data, leptin protein and gene expression, and visfatin as covariates. Obesity, higher TG, TC, LDL, leptin protein and gene expression, and lower HDL, and C-peptide levels were associated with uncontrolled diabetic patients in univariable analysis. Nevertheless, taking significant covariates in univariable analysis into multivariable analysis showed that lower C-peptide levels were suggested to be an independent predictor for uncontrolled diabetic children. So, according to our results, neither leptin nor visfatin can predict uncontrolled diabetes mellitus, but C-peptide does.

Table (IV): Regression analysis for prediction of T1DM in children.

	Univariable				Multivariable			
	p	OR	95% CI		*p*	OR	95% CI	
Age	0.838	1.008	0.936	1.085				
Gender	0.726	0.928	0.61	1.411				
Obesity	**0.008**	1.866	1.178	2.957	**0.036**	5.970	2.662	13.390
TG	**<0.001**	1.021	1.011	1.032	0.563	1.001	0.999	1.002
Cholesterol	**<0.001**	1.033	1.021	1.044	**0.040**	1.597	1.194	2.999
HDL	**<0.001**	0.739	0.654	0.835	0.739	1.001	0.995	1.007
LDL	**<0.001**	1.027	1.017	1.037	0.063	1.003	0.997	1.006
TNFα	**<0.001**	1.356	1.221	1.506	0.898	1.002	0.996	1.005
C- peptide	**<0.001**	0.757	0.672	0.852	**<0.001**	0.954	0.935	0.973
Leptin	**<0.001**	2.251	1.676	3.024	**0.037**	1.597	0.992	2.003
Visfatin	**<0.001**	0.610	0.479	0.776	**<0.001**	0.994	0.991	0.997

OR, odds ratio; CI, confidence interval; logistic regression analysis was used.

We implemented logistic regression analysis to predict diabetes occurrence within all studied cases, using age, gender, obesity, laboratory data, leptin protein and gene expression, and visfatin as covariates. Obesity, higher TG, TC, LDL, TNF-α leptin protein and gene expression, lower HDL, C-peptide, and visfatin levels were associated with diabetes mellitus development in univariable analysis. Nevertheless, taking significant covariates in univariable analysis into multivariable analysis showed that only obesity, lower C-peptide, higher TC,visfatin, and leptin levels were considered independent predictors for diabetes mellitus development in children. We did not find significant differences in total cholesterol and C-peptide among different studied BMI groups. Hence obesity, leptin, and visfatin may be considered more potential independent predictors for T1DM **Table (IV).**

4. Discussion:

In this study, BMI was highly significantly elevated in T1DM patients compared with healthy controls. In a previous study, researchers stated that diabetic patients are obese during their early adulthood more frequently than the normal population and are marked by increased body fat mass [20]. This agrees with our findings concerning the number and percentage between obese, overweight, healthy weight groups, and control groups. The prevalence of obesity has elevated rapidly in patients with T1DM than in the normal subjects. In comparison, increased insulin therapy, shortage of physical activity, and development of double diabetes demonstrate some mechanisms that explain weight gain in patients with T1DM [21].

Patients showed a highly significant increase in HbA1c, glucose, TG, TC, LDL, TNF-α, and a highly significant decrease in HDL and C-peptide serum levels compared to the control group.In another study, researchersfound that abnormal levels of BMI, high levels of TG, and decreased levels of HDL-C increase the risk of T2DM in the Chinese population [22]. Our finding of low C-peptide levels in the patient group agreed with previous research [23]. Low C-Peptide levels in patients with T1DM indicate the impairment of 70–90% of pancreatic beta cells.

TNF- α is produced by macrophages and monocytes with increased levels in adipose tissue and blood of T1DM individuals. Our study results showed an increased level of TNF-α in diabetic patients. This finding was in agreement with other researchers [24, 25], whose results showed TNF- α expression to be relatively high in obese human and animal subjects. In another study, researchers proposed that the increased level of pro-inflammatory cytokine in obese T1DM patients is related to obesity, not T1DM [26]. This increase may be due to the increased soluble TNF-receptors that might not be dissociated by the applied immunoassay. There was an increase in TNF-α levels by increasing weight, which was in

agreement with [27], in which researchers suggested the increase of TNF-α affects soluble intercellular adhesion molecule-1 by which it may stimulate vascular adhesion. High TNF-α levels are associated with dyslipidemia and increased blood pressure, increasing vascular disease risk.

Adipose tissue is a source of several adipocytokines that may contribute to vascular complications and have significant metabolic effects. One of these adipocytokines is visfatin. The results of this research showed a significant decrease in the levels of visfatin serum in the patient group compared to the healthy control group. This was in agreement with some studies [28, 29], in which researchers have shown that visfatin levels had significantly decreased in the patient group compared with healthy individuals and a significant association between HbA1C and visfatin levels. This may be due to the similarity of visfatin properties to insulin. In contrast, another study demonstrated that serum levels of visfatin increased with β-cell dysfunction in T1 and T2DM patients [30]. The reason for the impairment of visfatin signaling, dysregulation in biosynthesis, or response to hyperglycemia was the higher visfatin level in T2DM [31].

The explanation of the positive correlation of visfatin with glucose and HbA1c % may be the previous finding that the release of visfatin was improved by glucose in cultured adipocytes in human cultures *in-vitro* and hyperglycemia in normal subjects humans [32].

Moreover, the visfatin levels were positively associated with cholesterol, LDLc, and TG, while negatively associated with HDLc. Visfatin levels and lipid metabolism may be linked directly or indirectly. Furthermore, this relation may be a compensatory pathway for diabetic dyslipidemia.

Another research discovered significant differences in blood TG and cholesterol levels between diabetes and nondiabetic individuals [33]. These results agree with our outcomes that visfatin is correlated with higher blood lipid levels and may therefore be exploited to predict early atherosclerosis in Type 1 diabetic patients. Leptin plays an important role in diabetes pathogenesis, perhaps by inhibiting insulin production [34]. In this study, serum leptin levels were increased in type 1 diabetes patients than in healthy normal controls. This result was inconsistent with [35], in which researchers explained that this increase could be due to insulin therapyor, in other words, presumably due to peripheral hyperinsulinemia that usually results from exogenous insulin replacement. HbA1c may be considered the gold standard for assessing glycemic management [36]. In our study, serum leptin levels were positively correlated with HbA1c, and an increase was observed in leptin levels in the uncontrolled diabetic group than in a controlled diabetic group. These outcomes were inconsistent with [37], in which authors approved the significant positive correlation of leptin with HbA1c, which might be attributed to over substitution with insulin metabolic

mechanisms that consequently increase the release of leptin. They attributed the higher leptin levels glycemic control to T1DM of diabetic children (more concentrations of circulating HbA1c than individuals treated with insulin).

In this study, our results showed a significant positive correlation between obesity in diabetic patients and the mean serum leptin levels. This finding was consistent with [38], in which serum levels of leptin in schoolchildren with an age range of 9-13 years are shown to be correlated positively with BMI. Previous findings revealed that Obesity and BMI have a significant positive correlation with greater leptin levels in obese and non-obese diabetes patients (with higher BMI) than in normal healthy individuals, suggesting that it may be used as a biomarker of obesity [39]. Our results confirmed that there is a significant increase in leptin mRNA expression according to different body weights and so an association between leptin and BMI. This was in agreement with another study that concluded an elevation in leptin gene expression in the subcutaneous adipose tissue of obese children [40]. Also, this finding is consistent with [41], in which leptin mRNA level was found to be increased in overweight compared to normal-weight children. In addition, we performed a parallel protein quantification to confirm our mRNA results, and it was in the same consistency.

Our results showed considerable AUCs for leptin and visfatin for discrimination between patients and control groups. Also, in a previous study, researchersfound that the optimum cut-off point for leptin levels between the overweight diabetes group and the control group was determined to be 6.35 (ng/ml) and for visfatin to be 19.5 (ng/ml) [42]. T1DM patients with levels above or below the leptin and visfatin cut-off values, respectively, are at a greater risk of complications and should be constantly monitored.

Owing to the presence of several limitations in this study as a small sample size, we suggest further RCT experiments with larger samples and multicenter cooperation. Also, a risk group without undiagnosed diabetes and a large uncontrolled diabetic group subdivided into obese and non-obese are needed to get more integrated data. Furthermore, based on the obtained results that are shown a significant impact of both markers on the prediction of T1DM with high sensitivity and specificity and even with the acceptable significance of *p*-values.

5. Conclusion:

This study concluded that serum TNF-α, leptin, and leptin gene expression levels were increased, but serum visfatin level was decreased by increasing BMI. Visfatin is favorable over leptin as a predictor of T1DM in children. Also, the uncontrolled diabetic group was significantly associated with higher weight and BMI. The leptin gene was highly expressed in diabetic children compared to healthy controls. Also, uncontrolled diabetic children show

significantly higher leptin gene expression levels than controlled ones and are highly expressed in obese diabetic children compared to normal and overweight children. In addition, both markersLeptin, visfatin, and obesity may be considered independent predictors for DM. Further research, including samples higher in number and size and different diabetic categories, is recommended to check what marker is better for predicting T1DM.

We observed that there are significant elevations in leptin and TNF-α serum, while C-peptide and visfatin decreased significantly in diabetic individuals compared to healthy controls.The uncontrolled diabetic group showed significantly higher leptin and TNF-α for increased BMI individuals but lower C-peptide and visfatin than controlled diabetic individuals. While increasing leptin gene expression and TNF-α levels in uncontrolled diabetic obese groups but visfatin significantly decreased.

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Conflict of interest statement
All the authors declare that they have no conflict of interest in this work.

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