Evaluation of Serum Visfatin in Children and Adolescent with Type1 Diabetes Mellitus

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ABSTRACT
Background: Type 1 diabetes mellitus (T1DM) is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas. Visfatin is a ubiquitous intracellular enzyme, known as nicotine amide phosphoribosyl transferase (NAMPT) and pre-B-cell colony-enhancing factor (PBEF-1).
Objective: The aim of this study was to evaluate serum visfatin level in children and adolescent with type 1 diabetes mellitus (T1DM).
Patients and methods: The present study was a case-control study observation that was conducted in Pediatric Endocrinology Unit, Pediatric Ward, Zagazig University Hospitals. The study included 46 children; 23 with T1DM and 23 healthy age- and sex- matched children.
Results: In this study, we found that serum visfatin level in diabetic group is statistically highly significant lower than healthy group. The mean serum level of visfatin in healthy group was 19.53 ± 10.5 ng/ml, while in T1DM patients was 2.85 ± 2.09 ng/ml. The best cutoff of serum visfatin level in excluding T1DM was ≥ 3.6 ng/ml with area under curve 0.968 with sensitivity 91.3%, specificity 82.6%, positive predictive value 84%, negative predictive value 90.5%, positive likelihood ratio 5.25, negative likelihood ratio 0.11 and accuracy 87% ($p < 0.001$). The result showed that serum visfatin could be helpful in prediction of T1DM among children and adolescents with an accuracy 87%.
Conclusion: Serum visfatin level is lower in T1DM patients compared to healthy control. Visfatin play a role in early prediction and understanding the mechanism of its action in T1DM could lead to new therapeutic targets.
Keywords: Visfatin, Pediatric, Type1 Diabetes mellitus, Assessment.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a chronic illness characterized by the body's inability to produce insulin due to the autoimmune destruction of the beta cells in the pancreas [1]. Internationally the rates of T1DM are increasing. In Europe, Middle East, and Australia. T1DM is increasing by 2-5% per year [2]. T1DM is associated with a high morbidity and premature mortality. More than 60% of patients with T1DM do not develop serious complications over the long-term, but many experience blindness, end-stage renal disease (ESRD), and, in some cases, early death. The risk of ESRD and proliferative retinopathy is twice as high in men as in women when the onset of diabetes occurs before age 15 years [3].

Complications of T1DM include hypoglycemia from management errors, increased risk of infections, microvascular complications (retinopathy and nephropathy), neuropathic complications and macrovascular disease [3].

Adipose tissue secretes numerous bioactive peptides called 'adipokines'. Visfatin is a ubiquitous intracellular enzyme, known as nicotine amide phosphoribosyl transferase (NAMPT) and pre-B-cell colony-enhancing factor (PBEF-1). It is also identified as a novel adipocytokine [4]. Although the metabolic function of visfatin is still unknown, it appears that this newly identified adipocytokine might play an important role, similar to that of leptin, in the regulation of body weight, i.e. as an afferent signal reflecting excess body fat. The PBEF gene encodes a polypeptide of 491 amino acid residues. The secreted form of this polypeptide, i.e. visfatin, contains 465 residues and lacks the first 26 N-terminal residues of the PBEF gene product [5].

Recently, visfatin was isolated from visceral fat deposits. Like insulin, visfatin exerts hypoglycemic effects by interacting with the insulin receptor. The binding affinity of visfatin for the insulin receptor is similar to that of insulin, but it does not compete with insulin, suggesting that the two proteins interact with different receptor sites [6]. Visfatin, have an insulin-mimetic effects and lowers plasma glucose levels in mice via activating insulin receptor pathways [7]. Visfatin binds to the insulin receptor at a site distinct from insulin and stimulates phosphorylation of the insulin receptor, insulin receptor substrate 1 and 2 (IRS-1 and IRS-2), and the binding of phosphatidylinositol-3-kinase to IRS-1 and IRS-2 [8].

The aim of this study was to evaluate serum visfatin level in children and adolescents with Type 1 diabetes mellitus (T1DM).

PATIENTS AND METHODS

This study is a case-control study. It was conducted at the Pediatric Endocrinology Unit, Pediatric Ward, Zagazig University Hospitals through the period from March 2019 to March 2020.

The study included 23 patients with T1DM, their ages ranged from 1.25-16 years old (mean 8.1 years old), 12 of them were males and 11 were females. In addition, 23 healthy children served as a control group, their ages ranged from 3-12 years (mean age 6.8 years old), 11 of them were males and 12 were females.

Inclusion criteria: Children and adolescents with type 1 diabetes mellitus, and patient at age (1-18) years.

Exclusion criteria: T2DM, malignancy and immune-compromised patients, acute or chronic diseases, any other endocrine diseases, and any other renal pathology.

All diabetic children were subjected to the following:
1) **Full history taking stressing on** age, sex, duration of diabetes, and symptoms suggestive of microvascular diabetic complications including diabetic retinopathy, diabetic nephropathy and diabetic neuropathy.
2) **Complete physical examination including** general examination [Vital signs as pulse (rate/min), blood pressure measurement (systolic and diastolic)]. Anthropometric measurements [Weight (Kg): children were weighed wearing light clothes and without shoes to the nearest 0.5kg. Height / length (m): Length for children less than 2 years old is measured lying down, while standing height is measured for children age 2 years or older. Height was assessed without shoes, the child stands with his heels together stretching upward to full extent and the back is straight as possible. Body mass index was calculated as BMI= weight (kg)/height (m^2).

Laboratory investigations included Glycosylated haemoglobin (HbAlc), serum creatinine, BUN, serum albumin, urinary albumin, alb/creatinine ratio, GFR, and visfatin by ELISA: Determination of serum visfatin level by collecting samples of 3-5 ml venous blood that was left for coagulation at room temperature 10-20 minutes, then centrifugation for 20-min. At the speed of 2000-3000 r.p.m., then remove supernatant, if precipitation appeared centrifuge again. Plasma visfatin levels were measured using ELISA kits of human Visfatin (VF) ELISA Kit.

Chemical Method:
The quantity of the plates depends on the quantities of samples to-be-tested and the standards. It is suggested to duplicate each standard and blank well. Every sample was made according to the required quantity, and try to use the duplicated well as possible.

Ethical consent:
 An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis
Results of the presented study were collected and statistically analyzed using SPSS (statistical program of social science, (IBMO Corp., Armonk, NY, USA). The following statistical tests were used. Normality of numerical data distribution was tested using The D'Agostino-Pearson test. Description of quantitative variables as mean and SD. Description of qualitative variables as number and percentage. Unpaired student-t test was used to compare quantitative variables in parametric data (SD < 25% mean). Chi-square test was used to compare qualitative data. Ranked spearman correlation test to study the association between variables among each group. Receiver-operating characteristic (ROC) curve analysis was used to examine the value of continuous variables for prediction of binary outcomes. The DeLong method was used to compare the areas under the curve (AUC) for any pair of ROC curves. P value $\leq$ 0.05 was considered significant.

RESULTS
Table (1) showed a statistically non-significant difference between the studied groups regarding gender, age and BMI. 12 patients had been diagnosed with T1DM in the first visit (newly discovered), while 11 patients had T1DM for duration ranged from 3 weeks to 5 years with mean duration 2.56 ± 1.56 years.

Table (2) showed a statistically highly significant difference in HbA1c between the studied groups and a statistically significant difference between the studied groups regarding serum creatinine and eGFR. There was a statistically non-significant difference between groups regarding serum albumin, urinary albumin, BUN, Albumin/creatinine ratio, and presence of albuminuria.

Table (3) showed that there was a statistically highly significant difference between the studied groups regarding serum visfatin level (significantly lower in diabetic group).

Table (4) showed that the best cutoff of serum visfatin level in excluding T1DM was $\geq$ 3.6 ng/ml with area under curve 0.968 with sensitivity of 91.3%, specificity of 82.6%, positive predictive value of 84%, negative predictive value of 90.5%, positive likelihood ratio of 5.25, negative likelihood ratio of 0.11 and accuracy of 87% (p < 0.001).

Table (1): Comparison between the studied groups regarding demographic characteristics and BMI

	Diabetic group N=23 (%)	Control group N=23 (%)	X^2/Z/t	P
Gender				
• Male	12 (52.2)	11 (47.8)	X^2= 0.087	0.768
• Female	11 (47.8)	12 (52.2)		
Age (years)				
• Mean ± SD	8.11 ± 3.66	6.83 ± 2.15	Z= -1.83	0.067
• Range	1.25 – 16	3 – 12		
BMI (kg/m²)				
• Mean ± SD	16 ± 2.04	16.63 ± 1.29	t= -1.23	0.226

Table (2): Comparison between the studied groups regarding HbA1c and kidney function tests

kidney function test:	Diabetic group N=23(%)	Control group N=23(%)	Test X^2/Z/t	P
Albuminuria				
• Absent	19 (82.6)	23 (100)	Fisher	0.109
• Present	4 (17.4)	0(0)		
Urine albumin (mcg/ml)				
• Mean ± **SD**	26.97 ± 36.58	13 ± 3.69	-0.519	0.604
Serum albumin (g/dl)				
• Mean ± SD	3.99 ± 0.48	3.92 ± 0.14	0.671	0.508
BUN (mg/dl)				
• Mean ± SD	6.48± 3.61	5.58± 1.42	Z (-0.264)	0.792
Serum creatinine(mg/dl)				
• Mean ± SD	0.81 ± 0.13	0.66 ± 0.22	Z (-2.93)	**0.003**
eGFR(ml/min/1.73m)				
• Mean ± SD	126.89 ± 21.98	145.53 ±31.58	2.324	**0.025**
Albumin/creatinine ratio (mg/dl)				
• Mean ± SD	0.11 ± 0.02	0.12 ± 0.03	-1.361	0.18
HbA1c (%)				
• Mean ± SD	10.75 ± 0.48	4.96 ± 0.19	53.942	**<0.001**

Table (3): Comparison between the studied groups regarding serum Visfatin level

Serum visfatin level (ng/ml)	Diabetic group N=23	Control group N=23	Test Z	P
Mean ± SD	2.85 ± 2.09	19.53 ± 10.5		
Median	2.3	17	-5.438	**<0.001**

Table (4): Performance of serum visfatin level in differentiating healthy from diabetic patient

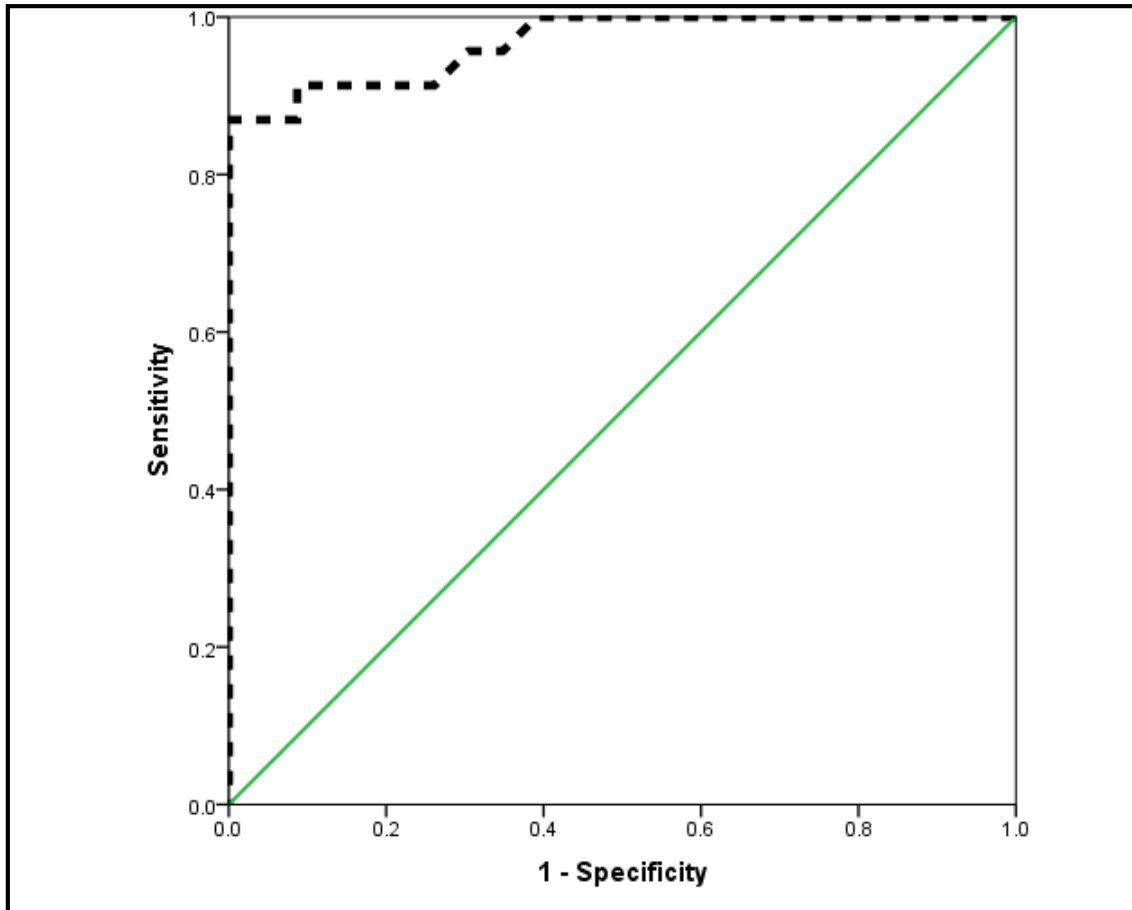| Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	+LR	-LR	Accuracy	P
≥ 3.6	0.968	91.3	82.6	84	90.5	5.25	0.11	87	**<0.001**

Fig. (1): ROC curve showing performance of serum visfatin level in differentiating healthy persons from diabetic subjects.

DISCUSSION

In this study, there was no significant difference between the two studied groups as regarding demographic and anthropometric measures; age, gender or BMI, which makes our study cross-matched. Our result is in agreement with **Alexiadou et al.** [9] who examined 100 subjects with T1DM and 52 healthy controls and found non-significant deference among both subjects regarding age, gender and BMI. Similarly, **Toruner et al.** [10] detected no statistical differences between the diabetic group and controls regarding age, sex and BMI in their study of 48 patients with T1DM and 26 healthy controls.

A statistically significant difference have been detected between studied groups regarding HbA1c, serum creatinine and eGFR. HbA1c and serum creatinine level were higher, while eGFR was lower in diabetic patients than healthy control. HbA1c is a simple blood test that gives average blood sugar level over the past two to three months [11]. In this study, the HbA1c was higher in T1DM than healthy group (our healthy group were free from chronic disease and diabetes). Making our result come with the American Diabetes Association in diagnostic criteria of T1DM. Our result is in agreement with **Bjornstad et al.** [12] who found that eGFR was lower in T1DM, while serum creatinine was higher in T1DM than healthy group. On the other hand our result disagrees with **Sur** [13] who studied 120 subjects (60 diabetics and 60

healthy) and found that no relationship in serum creatinine and eGFR between both groups (after excluding albuminuria).

Kidney function tests (eGFR, BUN and creatinine) in our diabetic patients are within normal, although GFR was lower and serum creatinine was higher in diabetic patients than in healthy. The normal finding of kidney functions maybe due to short course of the disease in our study ranged from 3 weeks to 5 years with mean duration 2.56 ± 1.56 years and exclusion of other risk factors like hypertension, chronic debilitating diseases and other metabolic diseases, which exacerbate diabetic nephropathy.

In this study, we find that serum visfatin level in diabetic group was statically highly significantly lower than healthy group. The mean serum level of visfatin in healthy group was 19.53 ± 10.5 ng/ml, while in T1DM patients was 2.85 ± 2.09 ng/ml. Our result is in agreement with **El Samahi et al.** [14] who revealed that T1DM patients had a significantly lower visfatin level compared to the healthy control. In addition, **Alexiadou et al.** [9] found that visfatin levels tended to be lower in patients with T1DM compared to healthy subjects. Also **Toruner et al.** [10] in their study on 48 T1DM and 26 healthy subjects reported that visfatin levels were lower in T1DM patients. On the other hand our result is in disagreement with **Hontzsch et al.** [15] who found that children and adolescents with T1DM had higher visfatin levels compared to healthy group.

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Also **AL-Suhaimi** *et al.* [16] measured serum visfatin among 28 Saudi pediatric volunteers consisted of 13 with T1DM and 15 healthy children. T1DM group had higher visfatin level than healthy group. Another study done by **López-Bermejo** *et al.* [17] reported that serum visfatin increase with β -cell dysfunction both in long-standing T1DM patient and T2DM.

The reason of controversial data about visfatin concentrations in T1DM is not clear. This discrepancy may in part, be due to different study populations as our diabetic patients were younger and had a shorter duration of diabetes and higher HbA1c levels than the other studies.

The best cutoff of serum visfatin level in excluding T1DM was ≥ 3.6 ng/ml with area under curve 0.968 with sensitivity of 91.3%, specificity of 82.6%, positive predictive value of 84%, negative predictive value of 90.5%, positive likelihood ratio of 5.25, negative likelihood ratio of 0.11 and accuracy of 87% (p<0.001).

The result showed that serum visfatin could be helpful in prediction of T1DM among children and adolescents with an accuracy of 87%.

CONCLUSION

Serum visfatin level is lower in T1DM patients compared to healthy control. Visfatin play a role in early prediction and understanding the mechanism of its action in T1DM that could lead to new therapeutic targets.

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