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Association between Vitamin D and Some Adipokines in Children with "Type I Diabetes Mellitus"

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ABSTRACT

Background: Visfatin and apelin are from adipokines which play a vital role in several physiological processes, such as homeostasis, and energy metabolism.

Aim: The aim of the work was to assess serum concentration serum apelin and visfatin concentrations in children with T1DM, their correlations to vitamin D and other clinical lab data, and to use these markers to anticipate T1DM in children.

Material and methods: 100 subjects with type 1 diabetes and 50 healthy age- and sex-matched subjects were included in our study. Concentrations of visfatin and apelin were measured by Enzyme-linked immunosorbent assay. Vitamin D levels were measured using auto-analyzer system (Mini Vidas, Biomerieux).**Results**: There were a significant increase in glucose, HbA1C, while decreased levels of C peptide, vitamin D and visfatin in patients than in healthy controls. Uncontrolled diabetic patients was significantly associated with higher weight BMI ,glucose, HbA1C, apelin and lower visfatin, C peptide and vitamin D when compared to controlled diabetic children

Visfatin level exhibit a positively significant correlations with age, glucose and a significant negative correlation with BMI, HbA1C, C-peptide, vitamin D and apelin. Aplein level showed significant positive correlations with BMI, and significant negative correlations with visfatin, and no significant correlation was present between apelin, vit.D, C-peptide, nor HbA1C.

Conclusion: apelin and visfatin could be considered as good biomarkers to predict diabetes. Also, the uncontrolled diabetic group was significantly associated with higher weight and BMI, HbA1C, and apelin, and with lower C-peptide.

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Introduction

T1DM is one of the most frequent disorders in children, affecting children of all ages. T1DM is becoming more prevalent, accounting for around 5% to 10% of adults with diabetes. Additionally, there is significant geographic diversity in occurrence worldwide. The highest rates are found in Finland and other Northern European countries, where they are about 400 times higher than in China and Venezuela, where the incidence is claimed to be the lowest. T1DM is most prevalent in non-Hispanic whites in the United States, impacting both females and males approximately equally [(1)]. T1DM develops in three phases. Stage I is without symptoms and is defined by normal levels of fasting glucose and glucose tolerance, as well as the existence of at least two pancreatic autoantibodies. Stage II diagnostic criteria include the presence of at least two pancreatic autoantibodies and dysglycemia, defined as impaired fasting glucose (glucose of 100 to 125 mg/dl) or impaired glucose tolerance (2-hour PG of 140 to 199 mg/dL) or a hemoglobin A1c of 5.7 to 6.4 percent. Individuals continue to exhibit no symptoms. Stage III is characterized by hyperglycemia with clinical signs and two or more pancreatic autoantibodies [(2)].

Diabetes mellitus diagnosis as reported by the American Diabetes Association (ADA) is based on evidence of impaired glucose metabolism, independent of the type of diabetes or the age at which it manifests [(3)]. T1DM can be diagnosed when a patient's random blood glucose is ≥ 200 mg/dl. A fasting blood glucose level of ≥ 126 mg/dl can also be used for diagnosis (fasting is defined as not eating or drinking anything for at least 8 hours, except water). A glycated hemoglobin (HbA1c) level of 6.5 percent, as determined by a National Glycohemoglobin Standardization Program-certified assay [(4)].

Vitamin D (25 (OH) D3) is belonged to fat-soluble secosteroids that is manufactured in the skin when cholesterol is subjected to ultraviolet B photons. Additionally, it can be gained by food or supplementation [(5)]. Vitamin D is related to calcium homeostasis, phosphorus levels, blood glucose metabolism and insulin resistance. Vitamin D receptors are located in most of human cells and tissues indicating that there are many extra-skeletal bioactivities of this molecule, especially in the immune system so its role in many disorders especially in autoimmune diseases should be investigated [(6)].

Vitamin D is vital for the etiology and avoidance of T1DM and plays a critical function in beta cell control in the pancreas. It primarily enhances insulin exocytosis by stimulating calcium-dependent endopeptidases, but it also enhances glucose tolerance and inhibits type 1 diabetes by acting as an efficient antioxidant and by reducing the expression of proinflammatory cytokines involved in T1DM pathogenesis, thus creating pancreatic β cells less prone to inflammation, resulting in decreased T-cell enrollment and infiltration and thus suppression of the autoimmune process [(7)].

Numerous research published in the last few years have disclosed increasing the frequency of vitamin D deficiency in individuals suffering from diabetes, implying that the insufficiency of this vitamin is correlated with the seriousness and recurrence of T1DM [(8)].

TIDM is related with metabolic irregularities and changes in the hormones produced by adipose tissue (adipocytokines or adipokines) and immunoglobulins. T1DM in children is marked by an evolved decline of glucose homeostasis caused by insulin secretion abnormalities, culminating in impaired glucose and other energy-producing fuels

metabolism. Glucose levels rise as a result of a deficiency of insulin-stimulated glucose disappearance and suppression of glucose uptake in skeletal muscle and adipose tissue. Adipocytokines are secreted by adipose tissue, including leptin, adiponectin, apelin, visfatin, and resistin, have been associated with development of insulin resistance [(9)].

One of the most important adipokines is apelin, it is secreted by adipose tissue and serves a variety of activities including insulin sensitivity by increasing cell sensitivity to insulin and delaying the onset of metabolic problems associated with obesity. Its concentration varies proportional to insulin resistance. Apelin plays a critical role in energy metabolism, glucose homeostasis, and the pathophysiology of type 1 diabetes because the white adipose tissue that produces apelin functions as an endocrine organ [(10)].

Visfatin is an adipokine so called because it is believed to be released by visceral fat. It has a molecular mass of 52 KDa and is encoded by a gene that has 491 amino acids. It is synonymous with pre-B cell colony-stimulating factor (PBEF). Visfatin was discovered to be secreted mostly by macrophages rather than adipocytes. There is adequate proof to suggest that visfatin is expressed by macrophages penetrating adipose tissue and is generated as a response to the inflammatory signals in this regard [(11)]. Visfatin effects are currently believed to be endocrine, paracrine, and autocrine. These autocrine activities may be critical in regulating the liver's insulin sensitivity [(12)]. The presence of visfatin was related with insulin resistance, implying that visfatin may have a role in the etiology of metabolic syndrome, impaired fasting glucose, impaired glucose tolerance, diabetes mellitus, and cardiovascular illnesses [(13)].

Thus, the purpose of the present work was to determine the serum apelin and visfatin concentrations in children with T1DM, their correlations to vitamin D and other clinical lab data, and to use these markers to anticipate T1DM in children.

Patients and Methods

Patients:

In our study, one hundred patients from Mansoura University Children Hospital, with T1DM, Diagnosing criteria was performed on the basis of the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines [(14)]. The patient's ages ranged from 6.5 to 12 years (9.6 ±3.1 years), they were 59 males and 41 females. Beside, 50 healthy volunteers (with similar ages and gender) and without any family history of Diabetes were included in the study. Exclusion criteria: children having any hepatic disease, vascular disease, renal disorder, acute or chronic infections, diabetic patients with acute illness, myocardial infarction were eliminated from this study.

The approval of this study was by The Ethical Committee of Faculty of medicine, Zagazig University, Egypt. **(ZU-IRB #6008/9-32018)**. A written consents were obtained from the parents of children.

Subsequent clinical and laboratory examinations were applied to all the participants:

The history of patients was taken thorough clinical examination with for age, sex, height, and weight. A portable free-standing stadiometer and an electronic scale (BS-8001, capacity: 130 kg respectively) was used for measurements of height and weight while wearing light cloths and no shoes. BMI was calculated as weight (kg) divided by the square of height (m). On the basis of BMI, they were classified to obese if BMI is ≥95th percentile; overweight if BMI is 95th≥ BMI ≥85th percentile and normal weight if BMI is 85th≥ BMI ≥5th percentile and this based on the specifications of the Center for Disease Control (CDC) growth charts [(15)].

Also, patients are classified according to HbA1C levels to controlled group (HbA1c ≤7.0 %) and uncontrolled group (HbA1c >7.0%).

Methods

Blood Sample and Biochemical investigations:

Fasting blood samples were collected in hospital lab. It was ensured that all patients and controls were fasting for (8-10) hours. Fasting blood glucose was determined enzymatically utilizing Spinreact diagnostic kits (San Antonio, Claret, Texas USA) .

Glycosylated hemoglobin (HbAlc) was measured using (MISPA i2, AGAPPE Diagnostics GmbH, Switzerland) which depends on nephelometric technique.

Vitamin D (25 (OH) D3) was measured using auto-analyzer system (Mini Vidas, Biomerieux, France) that depends on (Enzyme linked fluorescent technique). Quantification of C-peptide was performed on an immulite 2000 analyzer, Germany.

Serum Visfatin and apelin concentrations were determined by using ELISA technique (RayBiotech, Inc., Georgia, USA) and (MyBiosource, San Diego, CA) respectively following the manufacturer guidelines.

Statistical analysis

Data were analyzed using the statistical package of social sciences (SPSS) software version 21. Continuous variables were presented as mean ± SD (standard deviation). Student's t-test was used to evaluate the difference between the means of two sets of data. ANOVA test was used for comparison of means of more than two groups. Chi-square test was used to associate between categorical variables. P values < 0.05 were considered to indicate statistical significance.

RESULTS:

Patient showed significantly higher weight and height with (p=<0.001) compared to healthy individuals (Table1). When we compared the calculated BMI, we found a high significantly elevation ($p<0.001$) in patients than in healthy individuals.

We found that C-peptide significantly decreased in patient diabetic group than healthy group, and vitamin D levels in healthy controls were deficient and were insufficient in diabetic patients.

(Table 2) show that diabetic children exhibited a significantly higher glucose, HbA1C, while decreased levels of C peptide, vitamin D and visfatin than in healthy controls.

compared to the positive control group ($p<0.0001$).
On the basis of BMI we divided the patients into three groups (obese n=29, overweight n=32 and healthy weight n=39). We found a significant differences regarding the number and percentage of the three groups.

Visfatin deceased and apelin increases significantly with increased BMI (**<0.001**). No significant association was found in laboratory data including HbA1C, glucose, C-peptide or vitamin D between studied cases according to body weight.

Uncontrolled diabetic patients was significantly associated with higher weight BMI ,glucose, HbA1C, apelin and lower visfatin, C peptide and vitamin D when compared to controlled diabetic children. **(Table 3)**

Receiver operating characteristic (ROC) curve of Visfatin and Aplein levels for differentiation between patients and healthy controls. An ideal AUC was found for Aplein (AUC=1), visfatin showed an excellent AUC (= 0.997). Cut off values and performance characteristics are shown in (Table 4).

Comparing AUCs of Visfatin and Aplein levels for differentiation between patients

and controls, revealed that no significant differences were found between the two markers as they showed closed discrimination power between patients and controls.

Visfatin level exhibit a positively significant correlations with age, glucose and a significant negative correlation with BMI, HbA1C, C-peptide, vitamin D and apelin. As shown in (Table 5).

Aplein level showed significant positive correlations with BMI, and significant negative correlations with visfatin, and no significant correlation was present between apelin, vit.D, C-peptide, nor HbA1C.

Logistic regression analysis for prognosis of type I diabetes mellitus was performed using obesity, age, laboratory investigations, and visfatin as covariates. Obesity, increased apelin decreased vit. D, C- peptide, and lower visfatin levels were accompanied with diabetes development when applying univariable analysis. While taking significant covaraiates in univariable analysis into multivariable analysis disclosed that higher apelin lower C peptide, lower visfatin, obesity and vit.D, levels could be used as independent determinants for diabetes mellitus arise in children.

DISCUSSION:

In our study, patients showed significantly elevated BMI than in normal healthy controls. And this was in agreement with other researchers who stated that diabetic patients are obese during their early adolescence more regularly than the normal individuals and they are characterized with increased body fat mass [(16)]. This matches with our finding concerning the number and percentage between obese, overweight, healthy weight groups, and control groups. The currency of obesity has elevated rapidly in diabetic patients more than in the normal subjects. In comparison, increased insulin medication, shortage of physical exercises, and development of double diabetes demonstrate a probable explanation for obesity in patients with T1DM [(17)].

In patient group, glucose and HbA1C increased significantly, while C-peptide decreased significantly and this was in accordance with crisman et al [(18)] who found low C-Peptide levels in diabetic patients with.

There is a crucial role of cytokines and adipokines that are produced from adipose tissue as they participate in the autoimmune process by bringing up β cells destruction, and they may be considered prospective biomarkers for the disease progression.

Visfatin is one of these adipocytokines. Diabetic patients exhibited a significantly decreased visfatin levels than healthy controls. This was in accordance with Alexiado et al [(19)] in which they reveal the decreased visfatin levels in the patient group in comparison with normal subjects and a significant correlation between HbA1C and visfatin concentration. This may be due to the likeness of visfatin function to insulin. In contrast, another study indicated that serum levels of visfatin increased with β-cell dysfunction in T1DM patients. The reason for the impairment of visfatin signaling, dysregulation in biosynthesis, or response to hyperglycemia was the higher visfatin level in T2DM [(20)].

Our results showed considerable AUC for visfatin to differentiate between patients and control groups. Also, in a previous study, researchers found that the optimum cut-off for visfatin between the diabetic patients with overweight and healthy controls was determined to be 19.5 (ng/ml) [(21)]. T1DM patients with levels above or below visfatin cut-off value, are at a greater risk of complications and should be constantly monitored.

Apelin is an adipocytokine that is produced by human and mouse

adipocytes. Apelin synthesis and secretion from the adipose tissue are regulated by insulin. In our study we found an elevation in the levels of apelin in patient group when compared to healthy controls. The deficiency the insulin synthesized endogenously and secreted in diabetic patiens is accompanied with high levels of apelin and this was in agreement with Alexiadou et al [19] who showed that there were an increase in apelin levels in diabetic patients when compared with normal controls.

In our results, apelin levels were high significantly correlated with BMI, and this was in accordance with Du et al. [22] who found that apelin levels may be associated with obesity. The explanation of the elevated apelin concentrations in patients may be due to a challenge to make up for insulin deficiency likewise that the increased apelin levels in obesity or T1DM could possibly try to get control of insulin resistance and substitute the relative "lack" of insulin [23].

When we construct the (ROC) curve of apelin to differentiation between patients and healthy controls we found a perfect AUC for Apelin (AUC=1) so, we can state that the elevation of apelin levels above the cut off value may participate in the development of diabetes.

There are different studies displayed the presence of any correlation between vitamin D levels and the onset of T1DM, in spite of that some studies do not authenticate these results. In the present work, we found a significant difference between vitamin D levels in patients when compared to healthy controls. Vitamin D concentration was deficient in healthy and insufficient in diabetics and this was in agreement with a study in North America in which there is a decrease in vitamin D level in patients [24].

The role of vitamin D in T1DM pathological process may be by reducing lymphocyte proliferation and cytokine production by its immunomodulatory effect (25).

In our study, we investigate the correlation of vitamin D and the studied adipokines (visfatin and apelin) there was a negative correlation with visfatin and this is in agreement with Zang et al [26] who observed a decrease in visfatin levels in women taking vitamin D supplementation also observed a negative correlation between vitamin D and visfatin.

We couldn't discover any corporation between vitamin D and apelin in the present work.

Conclusion

We concluded an elevation in the levels of apelin, but serum visfatin, vitamin D, C-peptide levels was decreased by increasing BMI. We can predict the probability of T1DM occurrence in children using apelin and visfatin as a good biomarkers for the disease prediction. Also, the uncontrolled diabetic group was significantly associated with higher weight and BMI, HbA1C, and apelin, and with lower C-peptide.

Visfatin, apelin, lower C-peptide, vitamin D and obesity may be considered independent predictors for diabetes type 1. Further research, including samples higher in number and size and different diabetic categories, is recommended to check what marker is better for predicting T1DM.

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Table (1): Differentiation of demographic data between patients and healthy controls

		healthy controls	Patient group	*p*
Age (years)	**mean±SD**	9.5 ± 2.5	9.6 ± 3.1	0.841^{T}
Males	**N, %**	29, 58%	59 , 59%	0.726^{C}
females	**N, %**	22, 44%	41, 41%	
Weight (Kg)	**mean±SD**	39.2 ± 8.8	46.5 ± 11.1	**$<0.001^{T}$**
Height (cm)	**mean±SD**	142.9 ± 14.1	92.0 ± 30.4	**$<0.001^{T}$**
BMI (Kg/cm^2)	**mean±SD**	19.0 ± 2.4	20.8 ± 3	**$<0.001^{T}$**
Healthy weight	**n , percentage**	31, 62%	39, 39%	**0.022^{C}**
Overweight	**n , percentage**	12, 24%	32 , 32%	
Obese	**n , percentage**	7, 14%	29 , 29%	

Where, SD refers to "standard deviation", C refers to "Chi square test, and T refers to "independent t test".

Table (2): Differentiation of laboratory data between patients and healthy controls

	healthy group	Patient group	*p*

	Mean ±SD	**Mean ±SD**	
Glucose (mg/dL)	89.6±11	205±31	**<0.001[T]**
HBA1C (%)	4.4±0.8	8.0±1.6	**<0.001[T]**
C- peptide	3.8±0.8	0.5±0.2	**<0.001[T]**
Vitamin D	28.8±3.1	19.6±2.7	**<0.001[T]**
visfatin	28.7±4.4	11.3±3.6	**<0.001[T]**
Apelin	258.3±14	396.2±17.8	**<0.001[T]**

SD, standard deviation; T, independent t test

Table (3): Differentiation of age, gender, and anthropometric data between controlled and uncontrolled diabetic cases.

		Controlled N=46		**Uncontrolled N=54**		***p***
Age (years)	**mean±SD**	9.4±2.5		9.7±3.1		0.569[T]
Males	**N, %**	28	60.9%	31	57.4%	0.726[C]
females	**N, %**	18	39.1%	23	42.6%	
Weight (Kg)	**mean±SD**	43.0±7		48±11.0		**0.043[T]**
Height (cm)	**mean±SD**	146.1±8		149±11		0.830[T]
BMI (Kg/cm^2)	**mean±SD**	20±2		21±3		0.092[T]
Glucose (mg/dL)		**196.1±26.7**		**213.6±32.4**		**0.005[T]**
HbA1C (%)		6.7±0.4		9.1±1.3		**<0.001[T]**
C- peptide		**0.7±0.2**		**0.3±0.1**		**<0.001[T]**
Vitamin D		20.4±3.0		19.0±2.3		**0.007[T]**
Visfatin		**11.9±3.9**		**10.8±3**		**0.248[T]**
Apelin		384.1±11.2		406.5±15.9		**<0.001[T]**

SD, standard deviation; T, independent t test.

Table (4): The ROC curve of Visfatin and Aplein levels for differentiation between patients and healthy controls.

	Visfatin	Aplein
AUC	0.997	1
Cut off	<19.557	322.5
Sensitivity (%)	97	100
Specificity (%)	100	100
PPV (%)	100	100
NPV (%)	94.3	100
Accuracy (%)	98.0	100
P²	-	0.935

Where AUC refers to" area under curve"; PPV, refers to"positive predictive value"; NPV, refers to "negative predictive value". And p2, means comparison between AUC of apelin versus visfatin AUC.

Table (5): Differentiation of Visfatin levels with other parameters in all patients

	Visfatin	
	r	***p***
BMI	-0.440	**<0.001**
Glucose	0.261	**0.009**
HbA1C	-0.413	**<0.001**
C- peptide	-0.286	**0.004**
Vit. D	-0.487	0.391
Apelin	-0.336	**0.001**

Table (6): Differentiation of Aplein levels with different parameters in diabetic patients.

	Aplein	
	r	***p***
Age	-0.242	**0.015**
BMI	0.360	**<0.001**
Glucose	0.110	0.278
HbA1C	0.022	0.830
C- peptide	0.186	0.065
Vit. D	0.039	0.703
Visfatin	-0.336	**0.001**