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Role of Apelin in Egyptian Children with Type 1 Diabetes Mellitus

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Abstract:

Background: Adipose tissue yields many adipocytokines that modulate insulin sensitivity and play an essential role in the pathogenesis of diabetes. Apelin is a multifunction neuropeptide, involved in the regulation of food intake, cell proliferation and angiogenesis. This study aimed to investigate the role of apelin serum levels in children with type 1 diabetes mellitus (T1DM) and its relations with obesity and with some biochemical parameter serum levels. Methods: One hundred Egyptian children with T1DM and 50 healthy controls were involved in this study. Blood samples were withdrawn after 10 hours fasting for biochemical investigations of serum glucose, glycosylated hemoglobin (HbA1c), and apelin. Results: This study revealed that apelin concentration were higher in patients group comparing with control group, there is a significant increase in apeline by increasing weight. Conclusion: The increase of serum apelin in children may contribute to development of diabetes. These variations can be used as a specific marker in prediction of TIDM.

Key words: Diabetes type 1(T1DM); apelin; children.

Introduction:

Type 1 diabetes mellitus (T1 DM) is considered one of the most common autoimmune diseases in children (5–10% cases), one of its characteristics is the destruction of beta cells of the pancreas, resulting in absolute insulin deficiency **[1]**. Loss of tolerance to self-antigens (central and peripheral tolerance defect) is related to the destruction of pancreatic beta cells in T1 DM **[2]**.

Glucose concentrations rise due to lack of insulin-stimulated glucose disappearance, and suppression of glucose utilization in skeletal muscle and adipose tissue. The surplus glucose that is present in the blood reacts with hemoglobin to form glycated hemoglobin (HbA1c) in a non-enzymatic glycation pathway. Recently, HbA1c as introduced was an additional diagnostic criterion for diabetes and pre-diabetes **[3]**.

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Also, T1DM is usually accompanied by alterations in lipid metabolism, enhanced hyperglycemia-mediated oxidative stress, endothelial cell dysfunction, and apoptosis **[4]**.

Adipose tissue yields many adipocytokines that modulate insulin sensitivity, such as apelin, leptin, TNF, adiponectin, resistin,apeline, visfatin and IL-6. It was found to play an essential role in the pathogenesis of diabetes, insulin resistance, atherosclerosis and inflammation, regulation of energy metabolism, immune function, angiogenesis, and neuroendocrine function**[5]**. These proteins can act locally, through autocrine/paracrine mechanisms, or systemically, through endocrine effects **[6]**. Apelin is a ubiquitous, novel bioactive peptide originally secreted from white adipose tissues and many other tissues as stomach. Apelin is a multifunction neuropeptide. It has been shown to be involved in the regulation of cardiovascular and fluid homeostasis, food intake, cell proliferation and angiogenesis. The relation between apelin levels and glucose concentrations and insulin sensitivity prove that apelin may participate in the pathogenesis of diabetes **[7]**.

This study aimed to investigate the role of apelin serum level in children with type 1 diabetes mellitus and its relations with some biochemical parameter level and obesity.

Patients and methods:

i. Patients

One hundred children from Mansoura University Children Hospital, with diabetes mellitus type I were included in this study, and they were diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) **[8]**. The patients are 59 males and 41 females, with ages ranging from 6.5 to 12 years (9.6 ±3.1 years). In addition, 50 healthy children (with matched ages and gender) with no family history of Diabetes were included in the study.

This study was approved by The Ethical Committee of Faculty of medicine, Mansoura University, Egypt. A written consent was taken from parents of children.

ii. Methods

Patients and controls were subjected to the following clinical and laboratory investigations:

1- Patient history taken thorough clinical examination with special stress on age, sex, height and weight.

2- Determination of glucose, HbA1c, after overnight fasting for 8-10 hours.

Height and weight were measured using a portable free-standing stadiometer and an electronic scale (BS-8001, capacity: 130 kg respectively). Participants were measured wearing light cloths and no shoes. Weight was recorded to the nearest 0.1 kg whilst height was recorded to the nearest 0.5 cm. BMI was calculated as weight (kg) divided by the square of height (m). Based on the Center for Disease Control (CDC) growth charts, they divided to obese, overweight, and normal weight**. [9]**.

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Fasting blood glucose levels evaluated enzymatically using Spinreact diagnostic kits (San Antonio, Claret, Texas USA) . Glycosylated heamoglobin (HbAlc) was measured with nephelometric technique (MISPA i2, AGAPPE Diagnostics GmbH, Switzerland).

Serum apelin levels were determined by commercially available enzyme linked immunosorbent assay (MyBiosource, San Diego, CA) according to the manufacturer instructions.

iii. Statistical analysis

Statistical analysis was done 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 group showed heavier weight (p<0.001) with similar height (p=0.145) when compared to control group (Table1). The calculated BMI was found to be highly significantly raised (p<0.001) in type 1 DM patients as compared to controls.

Table (1): Comparison of anthropometric data between patient and control groups.

	Control group N=50	Patient group N=100	p
Weight (Kg) mean±SD	39.2±8.8	46.5±11.1	<0.001
Height (M) mean±SD	142.9±14.1	146.5±14	0.145
BMI (Kg/m^2) mean±SD	19.0±2.4	21.9±2.9	<0.001
Healthy weight N, %	N=31 62%	N=39 39%	<0.022
Overweight N, %	N=12 24%	N=32 32%	
Obese N, %	N=7 14%	N=29 29%	

Patients were divided into obese, overweight and healthy weight according to WHO (World Health Organization) Anthroplus Software for assessing growth of the world's

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children and adolescents**.** A significant difference was found concerning the number and percentage of these three groups, between patient and control groups (p<0.001).

Overweight and obesity were significantly associated with female gender. Nutritional status was not associated with age, as illustrated in table (2).

Table (2). Comparison of age and gender between studied cases according to nutritional status.

		Healthy weight N=39	Overweight N=32	Obese N=29	*p*
Age (years)	mean± SD	9±2.1	9,6 ±2.7	10,3± 3.3	0.193[A]
Males	N, %	29, 74%	17, 53%	13, 45%	0.036[C]
Females	N, %	10, 26%	15, 47%	16, 55%	

SD, standard deviation; A, ANOVA test; C, Chi square test.

Table (3) showed a highly significant elevation in glucose, HbA1c and apelin in diabetic group than normal control.

Table (3): Comparison of laboratory data between patient and control groups.

	Control group N=50	Patient group N=100	*p*
	mean±SD	mean±SD	
Glucose (mg/dL)	89±11.0	205±31.0	<0.001
HbA1c (%)	4.4±0.8	8.0±1.6	<0.001
Apelin (pg/ml)	258.3± 14	396.2±17.8	<0.001

Significant differences were found in the studied laboratory data between the three groups (obese, overweight and healthy weight) a significant elevation with the weight (p<0.001) was found in glucose, HbA1c and apelin serum level. Table (4).

Table (4): Comparison of laboratory data between studied cases according to BMI in studied patients.

	Healthy weight N=39	Overweight N=32	Obese N=29	*p*
	mean±SD	mean±SD	mean±SD	
Glucose (mg/dL)	196±23	206±30	216±37	0.137
HBA1C (%)	7.7±1.4	7.9±1.7	8.5±1.4	0.084

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Apelin (pg/ml)	389.9±16.4	391.9±15.2	409.0±16.5	<0.001

The cutoff point for apelin was 322.5, with 100% sensitivity and specificity for apelin, in the prediction of diabetes in children. Table (5).

Table (5): Area under ROC curve and performance criteria of apelin levels for discrimination between patients and control groups.

	Apelin
AUC	1
Cut off	322.5
Sensitivity (%)	100
Specificity (%)	100
PPV (%)	100
NPV (%)	100
Accuracy (%)	100

AUC: area under ROC curve, PPV: positive predictive value, NPV: negative predictive value.

AUC of apelin levels for discrimination between patient and control groups, revealed that it has an excellent discriminatory power for detection of the disease. Figure (1).

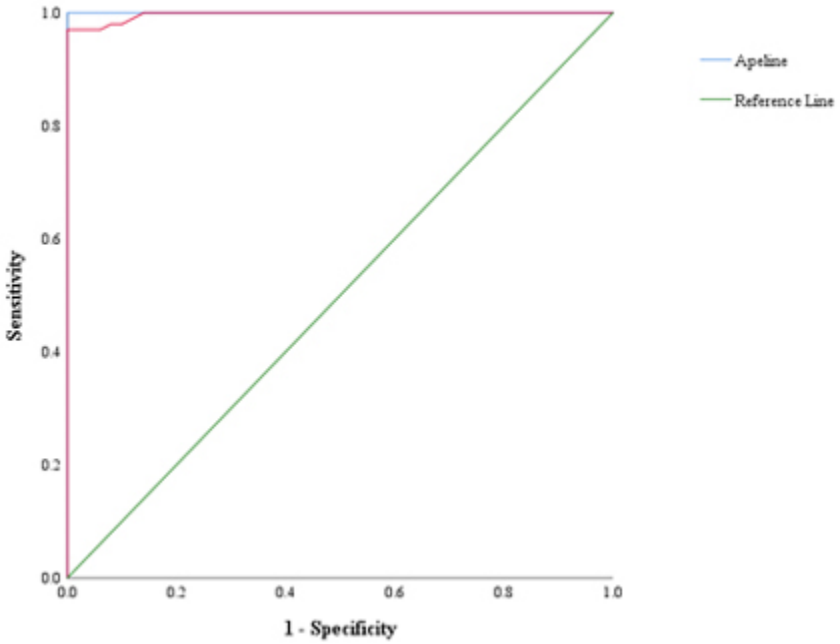

Figure (1).ROC curve and performance criteria of Apelin levels for discrimination between DM cases and control groups.

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Apelin level showed significant positive correlations with nutritional status, significant negative correlations with age. Otherwise, no significant correlations were found in Apelin levels with other parameters in all studied DM cases. (Table 6), figure (2).

Table (6): Correlation of apelin levels with other parameters in all studied DM cases.

	Apelin	
	r	*p*
Age	-0.242	0.015
Nutritional status	0.360	<0.001
Glucose	-0.110	0.278
HBA1C	-0.022	0.830

r, Pearson's correlation coefficient.

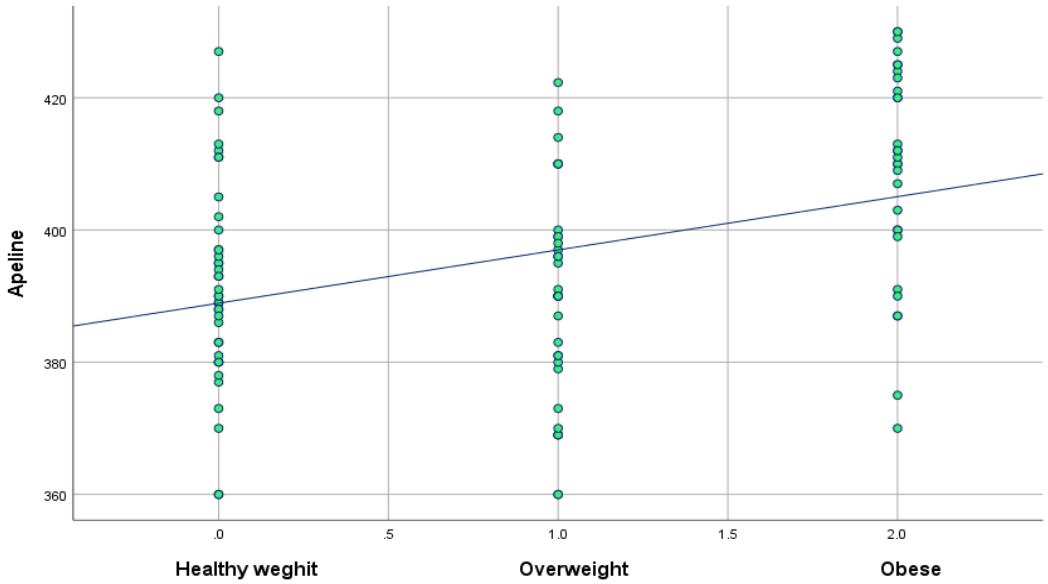

Figure (2). Correlation of apelin with Nutritional status in all studied cases.

Logistic regression analysis was conducted for prediction of DM occurrence, using age, gender, obesity, laboratory data, and apelin as covariates. Obesity, higher apelin, were associated with DM development in univariable analysis. However, taking significant covaraiates in univariable analysis into multivariable analysis revealed that only obesity,

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apelin levels were considered as independent predictors for DM development in children. Table(7).

Table (7). Regression analysis for prediction of DM development.

	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
Aplein	<0.001	1.002	1.001	1.003	<0.001	1.005	1.005	1.006

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

Discussion:

Type 1 diabetes 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. Onset most often occurs in childhood, but the disease can also develop in adults in their late 30s and early 40s **[10]**. Adipose tissue secretes many biologically active adipokines with diverse functions. **[11]**. Adipokines have several mediators such as adiponectin, pre-B cell colony-enhancing factor (PBEF) visfatin, apeline, leptin, resistin and retinol-binding protein-4 **[12]**. Apelin has been described as a new adipokine, produced and secreted by human and mouse mature adipocytes. Insulin may have a role in regulation of apelin synthesis and secretion from the adipose tissue. In the present study we showed that fasting plasma apelin concentrations were higher in patients with T1DM in comparison with healthy subjects. The lack of endogenous insulin synthesis and secretion in patients with T1DM is associated with high circulating apelin levels. Therefore, our data suggest that insulin may affect the expression of apelin in adults.

Alexiadou **[13]** showed that fasting plasma apelin concentrations were higher in patients with T1DM in comparison with healthy subjects. The lack of endogenous insulin synthesis and secretion in patients with T1DM is associated with high circulating apelin levels. In our study, apelin levels in serum were increased and associated with glucose homeostasis, and there was highly significant positive correlation between serum apelin and BMI in T1DM and this was with agreement with Du et al. **[14]** who suggesed that apelin levels may be associated with obesity. The higher levels of apelin found in patients with T1DM is due to a challenge to compensate for insulin deficiency in the same way that raised apelin levels in obesity or type 1 diabetes could possibly try to overcome insulin resistance and substitute the relative "lack" of insulin**[15]** . Alexiadou

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[13] also found that the lack of endogenous insulin synthesis and secretion in patients with T1DM is associated with up regulation of circulating apelin.**[16]** founded a vital role of apelin in the pathogenesis of obesity and obesity-related complications. Receiver operating characteristic (ROC) curve of Apelin levels for discrimination between DM cases and control groups. Perfect AUC was found for Apelin (AUC=1) Cut off values of apelin was 322.5 .We can state that increased serum apelin and in diabetic children may contribute to development of diabetes.

Conclusions:

The increase of serum apelin in children may contribute to development of diabetes and obesity. These variations can be used as a specific marker in prediction of TIDM. Apelin had significantly excellent discriminatory power. Apelin is a beneficial adipokine and is a promising therapeutic target in metabolic disorders as it had anti diabetic properties.

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