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A Study of Adiponectin in Children with Diabetes Mellitus

*Basma A. Ali,[1] Doaa M. Mahrous,[1] Ahlam M. Abdallah,[2] Mina Fikri[1]

دراسة الأديبونكتين في الأطفال الذين يعانون من مرض السكري

بسمة عبد المعز علي، دعاء محمد محروص، أحلام محمد عبد الله و مينا جرجس فكري قسم طب الأطفال، الكيمياء الحيوية

الملخص: الهدف: الأديبونكتين هرمون يفرز بواسطة النسيج الدهني ويفرز فقط من الخلايا الدهنيه و له علاقة بالبدانة والسكري و مضاعفاتهما. **الهدف:** تقييم مستوي الأديبونكتين في الأطفال المصابين بداء السكري بنوعيه الأول والثاني ومدي ترابطه بالإصابة بمضاعفات الأيض في مرضي السكري. **الطريقه:** أجريت هذه الدراسة بعيادة الغدد الصماء بمستشفى الأطفال الجامعي – جامعة المنيا خلال الفترة من أبريل 2011 حتى يوليو 2011 وشملت 314 طفل تتراوح أعمارهم من 2 إلى 18 سنة.وتم تقسيم الحالات إلى مجموعتين :المجموعة **الأولى**: تشتمل على 164 مريض تم تشخيصهم مسبقا كمرضى بداء السكري ثم قسمت هذه المجموعة إلى مجموعتين فرعيتين: – المجموعة الأولى أ: تشتمل على 142 مريض بداء السكري من النوع الأول.– المجموعة **الأولى ب**: تشتمل على 22 مريض بداء السكري من النوع الثاني والمجموعة **الثانية**: تشتمل على 150 طفل أصحاء ظاهريا ومتماثلين في السن والجنس .وقد خضعت كلتا المجموعتين إلي: اخذ التاريخ المرضى المفصل، الفحص الإكلينيكي والاختبارات المعملية وتشمل نسبة الهيموجلوبين السكري، وقياس مستوي الكولسترول والدهون الثلاثية وأيضا نسبة الأديبونكتين والسي–ببتيد) في الدم. **النتائج**: وجد أن نسبة الأديبونكتين بالدم لم تختلف كثيرا في الأطفال المصابين بداء السكري النوع الأول عن الأطفال المصابين بداء السكري النوع الثاني. و لكنها أعلى في مرضى السكري عن المجموعة الضابطة ومن حيث علاقة الأديبونكتين بالعوامل الأخرى وجد انه توجد علاقة تناسب طردية ذات دلالة إحصائية لمستوى الأديبونكتين بالدم مع محيط الوسط وفترة الإصابة بالمرض في الأطفال المصابين بداء السكري من النوع الأول وكذلك علاقة تناسب طردية ذات دلالة إحصائية لمستوى الأديبونكتين بالدم مع جرعة الأنسولين اليومية في الأطفال المصابين بداء السكري من النوع الثاني ومن ناحية أخرى تم إيجاد علاقة تناسب عكسية ذات دلالة إحصائية لمستوى الأديبونكتين بالدم مع كل من نسبة الكولسترول والسي–ببتيد بالدم و ضغط الدم الانبساطي في الأطفال المصابين بداء السكري من النوع الثاني الخلاصة : نتوقع من نتائج هذا البحث ان الأديبونكتين له أهمية في الحمايه من مضاعفات الأيض في مرضي السكري.

مفتاح الكلمات: السكري؛ الأديبونيكتين؛ مقاومة الأنسولينز؛ مصر.

ABSTRACT: ***Objectives:*** Adiponectin is a hormone produced by adipose tissue. It is secreted exclusively by adipocytes and appears to play a role in the pathophysiology of obesity, diabetes mellitus (DM), and its co-morbidities. The aim of this study was to assess adiponectin levels in diabetic children with type 1 DM (T1DM) and type 2 DM (T2DM), and to detect its prognostic role in them. ***Methods***: This study was undertaken from April to July 2011 at Minia University Children's Hospital, Egypt, and included 314 children aged 2–18 years divided into two patient groups. Group I consisted of 164 pre-diagnosed diabetic patients, further subdivided into Group Ia which included 142 patients with T1DM and Group Ib, 22 patients with T2DM; Group 2 included 150 apparently healthy children as a controls; they were age- and sex-matched to the diseased group. Patients were subjected to a thorough history taking, clinical examination, and laboratory investigations including assessment of HbA1c percentages, fasting C-peptide levels, lipid profiles and fasting serum adiponectin levels. ***Results***: Adiponectin levels did not differ significantly between patients with T1DM and T2DM, but it was significantly higher in diabetic patients than in the controls. In T1DM, adiponectin had positive significant correlations with the duration of the disease and waist circumference, while in T2DM, it had a positive significant correlation with the dose of insulin given and negative significant associations with diastolic blood pressure, cholesterol, and C-peptide levels. ***Conclusion***: The results of the study suggest that adiponectin can play a protective role against the metabolic complications of DM.

Keywords: Diabetes mellitus; Adiponectin; Insulin resistance; Egypt.

Advances in knowledge

- *Adiponectin, a hormone produced and secreted by adipocytes, is present in the blood in high circulating concentrations suggesting an important physiological role.*
- *An indirect regulator of glucose metabolism, adiponectin increases insulin sensitivity, improves glucose tolerance, and inhibits inflammation.*
- *Adiponectin is emerging as a risk factor for health problems such as diabetes, hypertension, and heart disease, yet there is very limited information on the distribution of this hormone in some populations, especially in children.*

[1]Paediatric and [2]Biochemistry Departments, Minia University, Minia, Egypt
**Corresponding Author e-mail: basmaelmoez@yahoo.com*

Application to Patient Care

- *The use of intensive insulin therapy in the management of type 2 diabetes mellitus might be protective as it improves insulin sensitivity by increasing adiponectin levels and normalising C-peptide, while adiponectin could also provide protection by affecting metabolic complications in the form of dyslipidaemia and hypertension.*

Egypt has one of the world's ten highest rates of diabetes mellitus (DM) and impaired glucose tolerance.[1] The ageing population together with socioeconomic and lifestyle changes has resulted in a dramatic increase in the prevalence of DM.[1] In DM, the improper regulation of glucose and lipid metabolism due to the lack of insulin leads to an increased lipolysis rate and decreased stored fat tissue, which is reversible after insulin administration.

Adiponectin is a hormone produced by the adipose tissue.[2] It is secreted exclusively by adipocytes, circulates at relatively high levels in the blood stream, and appears to play a role in the pathophysiology of obesity, DM, and its comorbidities.[2] This is because of its involvement in the regulation of carbohydrate and fat metabolism, as demonstrated in several animal and *in vitro* studies.[3] By acting through two distinct membrane receptors, adiponectin receptors 1 and 2 (which utilise 5-adinosine monophosphate-activated protein kinase phosphorylation, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha as key cell-signalling elements), increase hepatic and skeletal muscle sensitivity to insulin, enhance fatty acid oxidation, suppress monocyte-endothelial interaction, support endothelial cell growth, lower blood pressure, and moderate adipose tissue growth. The secretion of adiponectin can be suppressed by adipose factors, which are turned on once fat cell mass increases, such as cytokines, the adipose renin-angiotensin system, and increased oxidative stress. Inhibition of adiponectin secretion results in the loss of an array of mechanisms which, under normal conditions of fat cell homeostasis, provide protection from insulin resistance, DM, and atherosclerosis.[4] The aim of this study was to assess adiponectin levels in diabetic children with type 1 DM (T1DM) and type 2 DM (T2DM), and to detect their role as a prognostic factor.

Methods

This study included 164 patients with DM, 70 males (42.7%) and 94 females (57.3%); information on them was collected from April to July 2011 and they were classified as Group I. They had regular follow-up in the paediatric endocrinology outpatient clinic of Minia University Children's Hospital, Minia, Egypt. Informed consent was obtained from every subject after the study received the approval of the ethical committee of the Faculty of Medicine of Minia University. Participants were further subdivided into two groups. Group Ia (T1DM) consisted of 142 patients (86.6 %) with a mean of age of 10.9 ± 4.2 years. In this group, there were 64 males (45.1%) and 78 females (54.9%) with a mean duration of illness of 40.2 ± 12 months. Group Ib (T2DM) consisted of 22 patients (13.4 %) with a mean of age 14.5 ± 2.7 years. In this group, there were 6 males (27.3%) and 12 females (72.7%), with a mean duration of illness of 82.1 ± 39.6 months. Another 150 children (Group II) acted as a control group which was age- and sex-matched to the study group. Both groups underwent a thorough history taking. A clinical examination included anthropometric measurements, including weight, height, body mass index (BMI), and waist circumference (WC). Each measurement was taken as the mean of three consecutive readings. Laboratory investigations included a measure of glycosylated haemoglobin (HbA1c%), cholesterol, and triglycerides (TG).[5,6] Three ml venous blood samples were taken in the morning after 12 hours overnight using a complete aseptic technique. They were then centrifuged at 1000 rpm for 5 minutes. The sera were separated and stored at -20° C until an assay of C-peptide levels by enzyme-linked immunosorbent assay (ELISA) technique was performed. This was carried out using C-peptide ELISA kits (InterMedical, Villarica, Italy). Adiponectin levels were also determined by immunoassay technique using Quantikine (Human Total Adiponectin/Acrp30 Immunoassay) DRP300 kits (R&D Systems, Minneapolis, Minnesota, USA).7,8

Table 1: Comparison between the studied groups as regarding the fasting serum level of adiponectin (µg/ml)

Fasting serum adiponectin (µg/ml)	Group Ia (T1DM) (n =142)	Group Ib (T2DM) (n = 22)	Group II (Control) (n = 150)	*P* value			
				$P^{\dagger}$	$P^{\ddagger}$	$P^{\S}$	$P^{\P}$
Mean ± SD	9.5 ± 4.9	10.4 ± 5.7	4.9 ± 2.3	0.4	0.003*	0.002*	0.006*
Median	8.9	10.3	5.5				
Range	1.3–18.5	1.1–18.5	1.5–7.9				

*T1DM = type 1 diabetes mellitus; T2DM = type 2 diabetes mellitus; SD = standard deviation; * = significant; † = difference between Groups Ia and b; ‡ = difference between Groups Ia and II; § = difference between Groups Ib and II; ¶ = comparison between the studied groups.*

The data were coded and verified prior to data entry. All statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS), Version 19.0 (IBM, Inc., Chicago, Illinois, USA). For descriptive statistics, continuous variables were presented as mean followed by standard deviation (SD), and categorical variables were presented as frequency and percentage. In regards to analytical statistics, for qualitative data Pearson's chi-square test (χ^2) was used; for quantitative data, an independent samples t-test (for two groups) and a one-way analysis of variance (ANOVA) test and *post hoc* multiple comparisons with the least significant difference (LSD) equal variance assumed (for three groups), were used. Two-tailed partial correlation coefficients (r), adjusted for age, sex, and BMI were used to assess the relationships between adiponectin and other variables. A *P* value <0.05 was considered significant.[9]

Results

The study group (Group I) had significantly higher fasting serum levels of adiponectin than the controls (Group II) where *P* = 0.003 and 0.002, respectively. On the other hand, there was a non-significant difference between Groups Ia and b, where *P* = 0.4, although patients of Group Ib had the highest mean levels of adiponectin of all the studied groups [Table 1]. In the different laboratory investigations, the current study found that there were non-significant differences between Groups Ia and b in regards to the HbA1c percentage and triglycerides levels, while Group Ib had significantly higher levels of C-peptide and cholesterol than Group Ia (*P* = 0.0001 and 0.01, respectively) [Table 2]. Finally, concerning different correlations, we found that in Group Ia, there were significant fair positive correlations between mean adiponectin levels and both the duration of DM and WC, where r = 0.28 and *P* = 0.001 and r = 0.19 and *P* = 0.02, respectively. On the other hand, in Group Ib, there was a moderate positive significant correlation between mean adiponectin

Table 2: Laboratory investigations of Groups Ia and b

Laboratory investigations	Group Ia		Group Ib		*P* value
	Mean	SD	Mean	SD	
HbA1c (%)	7.5	1.4	8.1	0.62	0.06
C-peptide (ng/ml)	0.26	0.07	0.77	0.8	<0.001*
Cholesterol (mg/dl)	168.6	38.9	191.1	52.2	0.01*
Triglyceride (mg/dl)	131.2	205.3	140	54.2	0.8

*SD = standard deviation; HbA1c = glycosylated haemoglobin; * = significant.*

Table 3: Partial correlation coefficients adjusted for age, sex and body mass index between collected data and mean adiponectin in diabetic patients

	Group Ia		Group Ib	
	r	*P*	r	*P*
Insulin dose (IU/Kg/day)	-0.08	NS	0.66	0.002*
Duration of DM (months)	0.28	0.001*	0.37	NS
Waist circumference (cm)	0.19	0.02*	0.2	NS
DBP (mmhg)	-0.002	NS	-0.73	<0.001*
HbA1c (%)	0.03	NS	-0.32	NS
Cholesterol (mg/dl)	0.14	NS	-0.54	0.01*
Triglycerides (mg/dl)	0.1	NS	0.35	NS
C-peptide (ng/ml)	0.05	NS	-0.49	0.03*

*NS = not significant; * = significant; DM = diabetes mellitus; DBP = diastolic blood pressure; HbA1c = glycosylated haemoglobin.*

Grades of r: 0.00 to 0.24 (weak or no association); 0.25 to 0.49 (fair association); 0.50 to 0.74 (moderate association); ≥0.75 (strong association).

levels and the doses of insulin, and a moderate negative highly-significant correlation with diastolic blood pressure (DBP) (r = 0.66 and *P* = 0.002; r = -0.73 and *P* = 0.0001, respectively). Furthermore, there were negative significant correlations between the mean adiponectin level and both cholesterol and C-peptide, which displayed a moderate relationship with the former and a fair relationship with the latter (r = -0.45 and *P* = 0.01; r = -0.49 and *P* = 0.03, respectively) [Table 3].

Discussion

Several experimental studies have shown the anti-inflammatory and anti-atherosclerotic effects of adiponectin.[10] Furthermore, there is substantial evidence that adiponectin has protective effects against the development of atherosclerosis.[11] Therefore, the aim of our study was to assess the difference of adiponectin levels in children with T1DM and T2DM, and to detect its prognostic role.

In regards to the results of the current study, mean adiponectin levels in T1DM patients did not differ significantly from those in patients with T2DM (*P* = 0.4). This may have been due to the fact that our paediatric T2DM patients were on insulin therapy. Insulin downregulates adiponectin receptor expression, and insulin replacement could induce adiponectin resistance, making increased levels necessary to achieve physiological effects.[12] Also, several authors have observed that intensive insulin treatment increases circulating adiponectin levels, improving insulin sensitivity.[13,14] Moreover, the positive highly-significant correlation between the mean fasting serum adiponectin level and the dose of insulin in patients with T2DM in our study supports this result (r = 0.66 and *P* = 0.002). On the other hand, the mean adiponectin levels were significantly higher in diabetic patients than the controls. This result correlated with the results obtained by Furuta *et al.* who found that adiponectin levels increased in conjunction with β-cells dysfunction.[15] Also, Barnes *et al.* and Ljubic *et al.* found that adiponectin levels were significantly higher in diabetics than in controls.[16,17]

Concerning laboratory investigations, the current study found that there was an insignificant difference between patients in Groups Ia and Ib in regards to HbA1c percentage. On the other hand, Group Ib had significantly higher mean C-peptide levels than Group Ia. Furthermore, they had significantly higher cholesterol levels than group Ia. It is possible that poor glycaemic control among those with T2DM contributed substantially to the high lipid profile.[18] This result correlated with the results obtained by Dabelea *et al.* and Mayer-Davis *et al.*, who found that T2DM patients had higher C-peptide levels and cholesterol than T1DM patients.[19,20] Contrary to our results, Wadwa *et al.* found that there was a non-significant difference between T1DM and T2DM in regards to cholesterol levels.[21]

Concerning different correlations, in Group Ia mean serum adiponectin had a significant fair positive correlation with duration of DM. This result was in agreement with the result obtained by Lindström *et al.*[22] Also, there was a significant weak positive correlation between mean adiponectin levels and WC. This was possibly due to the fact that 93% of Group Ia were lean patients. On the other hand, in Group Ib there was a moderate positive significant correlation between mean adiponectin level and dose of insulin. This might be due to the fact that insulin therapy increased circulating adiponectin levels and decreased insulin resistance by increasing the metabolic insulin signal in human skeletal muscles.[13] Furthermore, there was a moderate negative highly significant correlation between adiponectin and DBP. This result correlated with the results obtained by Degawa-Yamauchi *et al.* in their study of African boys, Weiss *et al.* in their study of French adolescents, and Hassan *et al.* in their study of obese children.[23–25] This could possibly be due to the beneficial role of adiponectin in the management of endothelial dysfunction as adiponectin acts directly on vascular endothelial cells and exerts salutary effects on endothelial function through endolethial nitric oxide synthase (eNOS)-dependent and cyclooxygenase-2 (COX-2) dependent regulatory mechanisms.[26] Also, there was a moderate negative significant correlation between mean adiponectin and total cholesterol among Group Ib. This may have been due to the anti-inflammatory and anti-atherosclerotic effects of adiponectin.[27] Finally, there was a fair negative significant correlation between serum adiponectin levels and C-peptide. This could be explained by the adiponectin level increase in conjugation with β-cell dysfunction, which was estimated by fasting serum C-peptide.

Conclusion

Based on the results of this study, we concluded that there was a non-significant difference in the adiponectin levels between T1DM and T2DM, but that diabetic patients had significantly higher levels than the control group. The increase in circulating adiponectin concentrations in patients with T1DM appeared to be associated with a longer duration of illness and a greater WC. On the other hand, pancreatic β-cell function is a significant regulator for serum adiponectin concentrations in T2DM patients where serum adiponectin concentrations were associated with C-peptide. Also, in T2DM, adiponectin had a significant negative association with DBP and hypercholesterolaemia, suggesting its protective role against the metabolic complications of DM.

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