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CREATIVE STUDIO

Apelin, Nitric Oxide and Vascular Affection in Adolescent Type 1 Diabetic Patients

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Abstract

AIM: To evaluate the relationship of apelin and nitric oxide (NO) to endothelial dysfunction in type 1 diabetics.

PATIENTS AND METHODS: Sixty two type 1 diabetics and 30 healthy age and sex matched controls were included. Blood samples for apelin, NO, glycosylated hemoglobin (HbA1c), and lipid profile were collected. Albumin/creatinine ratio was assessed in urine. Flow mediated dilatation (FMD) via ultrasound was done.

RESULTS: The mean age of diabetics were 16.3 ± 1.5 yrs (14.0 – 19.0 yrs), and duration of disease, were 9.4 ± 2.9 yrs (5.0 – 16.5 yrs). FMD and FMD/nitrate mediated dilatation (NMD) ratio were lower in diabetics. NO was decreased, while apelin and albumin/creatinine ratio were increased significantly in diabetics. There was a positive correlation between apelin and HbA1c. On the contrary, NO had a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c and OxLDL.

CONCLUSION: Diabetic patients had endothelial dysfunction and high apelin level, with no related to each other. High level of apelin is associated with bad glycemic control. Obesity had no role to increase in apelin level. NO is related to diabetic nephropathy and atherosclerosis. We recommend a further large study to evaluate the relationship of apelin with endothelial dysfunction.

Introduction

Apelin, a recently described adipocytokine, is abundantly expressed in adipose tissue and produced in many body parts by the endothelial cells [1, 2]. It is synthesized as a prepropeptide then modified into smaller peptides with higher potency. It produces its effects through a cell surface G protein-coupled receptor called APJ, which is structurally similar to angiotensin receptor [3]. In obese persons with hyperinsulinemia, apelin levels are increased [4]. Levels are decreased in patients with dyslipidemia and newly diagnosed and untreated type 2 diabetes mellitus (T2DM) [5, 6].

Apart from insulin deficiency, insulin resistance is found in type 1 diabetes mellitus (T1DM), both at onset and course of the disease [7, 8]. Insulin resistance is also found in obesity making it a risk factor for T1DM in children in addition to type II DM [9-13]. Obese infants have higher risk for T1DM in chidhood [14]. This may be due to the harmful effects to the beta cells early in life as a result of the relative hyperinsulinemia induced by the increased demands in obesity [13, 15, 16].

There is endothelial dysfunction with increased cardiovascular risk in type 1 diabetes. L-Arginine is converted to nitric oxide (NO), which is an important mediator of vascular homeostasis due to its central role in the maintenance of the endothelial milieu [17, 18].

Cohen [19], reported endothelial dysfunction is the earliest event in the atherosclerotic process and Järvisalo et al. [20], found impaired FMD response is a common manifestation in children with type 1 diabetes and associated with high carotid artery IMT,

suggesting that endothelial dysfunction in with type 1 diabetics may predispose them to the development of early atherosclerosis.

We are aim to evaluate apelin and nitric oxide (NO) in type 1 diabetic patients and its relation to vascular affection as well as to evaluate the relationship between apelin and the glycemic balance.

Patients and Methods

Patients

The study included 62 adolescent patients with type 1 diabetes mellitus (DM) among those attending to the endocrine clinic, National Research Centre. The control group consisted of 30 age and sex matched healthy normal volunteers. Control group was the healthy friends or relatives of our patients.

Inclusion criteria: children with type 1 DM, duration of disease > 5 years, patients age > 14 and < 19 yrs old. We selected this young age group with short duration of diabetes firstly, to explore whether early atherosclerotic changes starts at this early age shortly after onset of diabetes or needs longer exposure to the diabetic milieu and secondly because in younger age group (< 14 yrs old) atherosclerotic lesions are expected to be in the form of microscopic intimal fatty streaks that is too minute to be resolved by ultrasonography.

Exclusion criteria were: patients during acute diabetic complications e.g. diabetic ketoacidosis (DKA) or hypoglycemia, patients suffering from cardiac diseases e.g. congenital, rheumatic heart, left ventricular dysfunction, patients on metformin or multivitamins and smokers.

Study design and protocol

It is a cross-sectional observational study done after obtaining approval from the ethical committee of the National Research Centre, Cairo, Egypt. Registration number is 11052. Written informed consent was obtained from all patients or their parents and controls after full discussion about the aim of the study. This study is a part of a project done in the National Research Centre for evaluation of cardiac, vascular and endothelial function in adolescent type 1 diabetic patients.

All the studied patients were subjected to: history taking including: age of patients, sex, age of onset of diabetes, duration of diabetes, type and dose of insulin therapy, family history of diabetes; and we asked about presence of any symptoms of cardiac, renal, neurological affection or presence of any type of autonomic dysfunction. We also asked about history of taking drugs other than insulin.

Clinical examination

I. Patients and controls were subjected to general, cardiac, chest and neurological examination.

II. Blood pressure was measured three times for patients and controls after 5-minute rest in the sitting position on both upper limbs with the use of automatic manometer (Omron M4 Plus, Omron Health care Europe, Hoof drop, and Holland). The mean value of the second and the third measurement was calculated. The measurements taken on the dominant limb were analyzed.

III. Anthropometric measurements in the form of weight, height, waist circumference (WC), and hip circumference (HC) were taken for each participant. The weight and height of the participants were measured up to 0.01 kg and 0.1 cm using a Seca Scale Standing Balance and a Holtain Portable Anthropometer (Holtain, Ltd, Crymmych, Wales, U.K.). Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured at the level of the umbilicus with the participant standing and breathing normally; hip circumference was measured at the level of the iliac crest, using non stretchable plastic tape to the nearest 0.1 cm. The waist / hip ratio and waist / height ratio (cm/ cm) were calculated. Each measurement was taken as the mean of three consecutive measurements, using standardized equipment [21, 22]. The landmarks, instruments used, and techniques followed were those recommended by the international biological program [21, 22].

Laboratory investigation

Simultaneously all patients and controls underwent the following tests:

All patients and controls underwent the following tests: For cholesterol measurements, venous blood was sampled after a 12-h fast. Serum total cholesterol was determined by a commercial kit (Boehringer-Mannheim, Germany) [23]. High-density lipoprotein (HDL) cholesterol was separated from the serum by precipitation of the other lipoproteins with a heparin/manganese procedure [24]. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation. The concentration of triglycerides(Tg) was measured in a TechnoCon AutoAnalyzer II (TechnoCon Instruments, Tarrytown, NY, USA).

Glycosylated hemoglobin (HbA1c) was done every 3 months and the mean value was calculated per year. It was measured using high pressure liquid chromatography (Nichols Institute, Van Nuys, CA, USA) [25].

Screening for microalbuminuria was assessed

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in fresh morning urine samples by measuring albumin/creatinine ratio by enzyme linked immunosorbent assay (ELISA) kit provided by Orgentec Diagnostika, Gmbh (Mainz, Germany) [26].

Serum concentrations of oxidized low-density lipoprotein (OxLDL) were detected by commercially available solid phase two-site enzyme immunoassay kit (Mercodia AB, Uppsala, Sweden). Measurements of the OxLDL levels in the sera were performed according to the recommendations of the manufacturer. The intra and interassay coefficients of variations were 5.5% – 7.3% and 4.0% – 6.2%, respectively, and the sensitivity was < 1 mU/L.

Nitric oxide production in sera was measured using a nitric oxide colorimetric assay kit (Roche cat No. 11756281001). Sera was irradiated and then filtered in a 10,000 kd MWCO column (Satorious, Vivascience cat No. 13239-E). The assay was conducted as per the manufacturer's instructions using a NO control with a standard curve plotted and samples were measured at a 550 nm wavelength. Serum apelin-12 concentration were measured using a commercial enzyme – liked immunosorbent assay (Phoenix Pharmaceuticals Inc. Burlingame,CA,USA).

Flow mediated dilatation (FMD)

All imaging studies were performed by the same vascular sonographer & the same ultrasonographic machine using (General Electric medical ultrasonographic machine model: Vivid 7 Pro, GE Vingmed ultrasound AS-Nl90, Horton-Norway equipped with 7.5–10 MHz linear-array transducer) after the published protocols [27-29]. With the subject lying in the supine position, ECG electrodes were placed on the chest; the machine automatically measured and recorded results of the electrocardiogram. All measurements were made at end diastole to avoid possible errors resulting from variable arterial compliance. A sphygmomanometer cuff was placed on the proximal right arm. The right brachial artery images were obtained 3 cm proximal to the elbow crease using B-mode imaging in the longitudinal plane of the artery. A baseline image was acquired using a resolution box function to magnify this part of the artery. Blood flow was estimated by the pulsed Doppler velocity signal obtained from a mid-artery sample volume. The cuff was inflated to 100 mm Hg above the systolic pressure to occlude arterial flow for 5 min. The cuff was then deflated, and the longitudinal image of the brachial artery was recorded immediately & continuously for 60 seconds after cuff deflation for greatest response guided by the hyperemic flow detected by pulsed Doppler. Flow-mediated dilation (FMD) was assessed by measurement of the greatest brachial artery diameter that was detected at 60 seconds after release of the cuff in most cases. The subject then had a rest for 30 min, after which a sublingual dose of nitroglycerin

tablet (Dinitra, isosorbidedinitrate 5 mg manufactured by Egyptian int. pharmaceutical industries co., tenth of Ramadan city, A.R.E., under license of RHONE POULENC, PARIS FRANCE) was then administered, and the brachial artery response (endothelium-independent dilation) was assessed by imaging the artery continuously for 3 min after the nitroglycerin dose for greatest response.

Measurements of the brachial artery luminal diameter were performed on-line at end-diastole, coinciding with the onset of the R-wave on the ECG. For each phase (baseline, endothelium-dependent dilation, and endothelium-independent dilation), three brachial artery diameter measurements were obtained manually online with electronic calipers and averaged from the longitudinal image by identifying the lumen-intima interface. The largest reading for FMD post-ischemia {100 × [diameter (1 min)–diameter (basal)]/diameter (basal)} was used to represent spontaneous endothelial function. In addition, nitroglycerine-mediated dilatation (NTG) {100 × [the largest reading of diameter (after sublingual isosorbidedinitrate–diameter (basal)]/diameter (basal)} was assessed. The diameter percent change caused by endothelium-dependent flow-mediated dilatation (%FMD) and non-endothelium dependent dilatation (%NMD) were expressed as the percent change relative to that at the initial resting scan.

Significant endothelial dysfunction was defined as FMD < 10% and NMD > 10% [30]. In order to increase the sensitivity and specificity of the technique for endothelial dysfunction, FMD over NMD of the brachial artery < 0.70 defined endothelial dysfunction [31].

Statistical Analysis

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) program version 15.0 (Chicago, Illinois, USA). t –test or Mann Whitney-U (for none symmetrically distributed data) for independent variables was done. Pearson's correlation was also done.

Results

Our research included 62 type1 diabetic patients (31 females and 31 males) and 30 normal controls (15 females and 15 males). Their mean age were 16.3 ± 1.5 yrs and mean duration of diabetes were 9.4 ± 2.9 yrs. HbA1, albumin/.creatinine ratio, cholesterol, Tg, LDL, OxLDL and apelin were significantly increased, in the contrary, nitric oxide, FMD, and FMD/NMD were decreased significantly in diabetics (Table 1).

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Table 1: Comparison between demographic, laboratory data, carotid intimal medial thickness and resistivity index of diabetic patients and controls

Variables	Patients		Controls		P-value
	Mean	SD	Mean	SD	
Age (yrs)	16.32	1.52	16.13	2.63	0.70
Anthropometric data:					
BMI (kg/m^2)	24.91	4.20	24.76	5.67	0.8
BMI (SDS)	24				
Waist circumference (cm)	83.60	9.39	84.78	12.25	0.60
HIP circumference (cm)	91.69	8.37	91.20	11.93	0.80
Waist / hip ratio	0.91	0.06	0.93	0.05	0.20
Waist / height ratio	0.51	0.06	0.52	0.08	0.90
Blood pressure:					
Systolic blood pressure (mmHg)	119.35	12.53	118.21	14.42	0.70
Diastolic blood pressure (mmHg)	81.94	9.20	78.57	6.51	0.05
Laboratory data:					
HbA1 (%)	9.55	1.90	5.43	0.65	0.0001
		28.4		10.7	
Albumin/ creatinine ratio (µg/ g creatinine)	78.33 ± 100.65 (5.8 – 384.2)		11.28 ± 4.23 (5.4 – 23.2)		0.0001#
Total cholesterol (mg/dl)	188.81	63.77	100.54	20.41	0.0001
Triglyceride (mg/dl)	103.46	78.29	68.89	28.39	0.03
HDL-c (mg/dl)	51.77	20.58	52.21	11.12	0.90
LDL-c (mg/dl)	118.66	47.53	62.50	19.88	0.0001
OxLDL (mg/l)	17.56	6.45	9.06	3.92	0.0001
Nitric oxide (µmol/l)	28.42	7.06	40.33	6.32	0.0001
Apelin	223.95	185.83	70.45	27.74	0.0001
Doppler on brachial artery					
Brachial artery at rest, mm	3.42	0.39	3.26	0.79	0.2
Brachial artery (Shill test) (FMD), %	10.35	7.16	15.64	10.77	0.005
Brachial artery after nitrate (NMD), %	14.48	6.89	18.14	10.77	0.1
FMD/NMD	0.82	0.66	2.26	6.29	0.03

t- test for independent variables; # Mann Whitney U test was used; Median, mean ± SD (range). BMI = body mass index; HbA1 = glycosylated hemoglonin; LDL = Low density lipoprotein; HDL = high density lipoprotein; OLDL = oxidized low density lipoprotein.

Apelin had a significant positive correlation with HbA1c (Table 2).

Table 2: Correlation between demographic, anthropometric, laboratory data and flow mediated dilatation and Apelin of diabetic patients

Variables	Apelin	
	r	P-value
Age (yrs)	0.14	0.30
Duration of diabetes (yrs)	-0.05	0.72
Systolic blood pressure (mmHg)	0.17	0.22
Diastolic blood pressure (mmHg)	0.21	0.12
BMI (kg/m^2)	-0.05	0.72
BMI (SDS)	-0.06	0.65
Waist/ hip ratio	0.15	0.28
Waist / height ratio	-0.03	0.81
HbA1 (%)	0.26	**0.04**
Albumin / creatinine ratio (µg/g creatinine)	-0.05	0.74
Cholesterol (mg/dl)	0.02	0.91
Triglycerid (mg/dl)	-0.03	0.84
HDL-c (mg/dl)	0.06	0.67
LDL-c (mg/dl)	0.07	0.64
VLDL (mg/dl)	0.42	0.10
oxldl (mg/dl)	-0.11	0.44
Nitric oxide	0.05	0.74
Doppler on brachial artery at rest (mm)	0.08	0.55
Doppler on brachial artery (Shill test) (FMD)(%)	-0.04	0.76
Doppler on brachial artery after nitroglycerine (NMD) (%)	0.17	0.22

On the other hand, NO had a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c and OxLDL (Table 3).

Discussion

In the current study, diabetic patients had higher HbA1c, albumin/creatinine ratio, lipid profile, OxLDL and lower NO, FMD and FMD/NMD. This is comparable with the study of Schulze el al., [32], who reported that the earliest functional atherosclerotic changes in the arterial wall is the endothelial dysfunction due to impaired endothelial release of nitric oxide detected by measuring flow mediated dilatation (FMD) of the brachial artery for measuring arterial diameter in response to increased flow.

Table 3: Correlation between demographic, anthropometric, laboratory data and flow mediated dilatation and nitric oxide of diabetic patients

Variables	Nitric oxide	
	r	P-value
Age (yrs)	0.04	0.75
Duration of diabetes (yrs)	-0.23	0.07
Systolic blood pressure (mmHg)	0.13	0.34
Diastolic blood pressure (mmHg)	0.13	0.31
BMI (kg/m2)	0.15	0.25
BMI (SDS)	0.18	0.17
Waist/ hip ratio	-0.02	0.90
Waist / height ratio	-0.09	0.49
HbA1(%)	**-0.30**	**0.02**
Albumin / creatinine ratio (µg/g creatinine)	**-0.55**	**0.0001**
Cholesterol (mg/dl)	-0.10	0.47
Triglycerid (mg/dl)	-0.16	0.22
HDL-c (mg/dl)	0.16	0.23
LDL-c (mg/dl)	-0.26	**0.05**
VLDL (mg/dl)	-0.43	0.07
OxLDL(mg/dl)	-0.32	**0.01**
Apelin (ng/ml)	0.05	0.74
Doppler on brachial artery at rest (mm)	0.08	0.53
Doppler on brachial artery (Shill test (FMD)(%)	0.05	0.73

Reduction of NO may be a result of either decreased production because of decreased activity and/.or reduced expression of eNOS, or low activity of NO, or a result of high degradation by high production of superoxide ions or reactive oxygen species [17, 33].

In the present study, NO showed a negative correlation with HbA1c, albumin/creatinine ratio, LDL-c, and OxLDL. NO in type 1 diabetics affect heart and kidney in the previous studies. Endothelial function affected by several factors associated with diabetes, including severity of hyperglycemia, duration of diabetes, and increase of advanced glycosylated end products, microalbuminuria and nephropathy [18].

In the present study, the attenuated FMD response in diabetic children coincide with the results of Wiltshire et al., [34] and Donaghue et al., [35], who studied flow-mediated dilation in diabetic children and demonstrated attenuated endothelial function in diabetic children compared with controls.

In our study, apelin is increased in diabetic patients with a positive correlation with HbA1c. On the other hand, it had no significant correlation with anthropometric measurement, lipid profile, NO and FMD.

Apelin mRNA is found in many tissues including central nervous system (CNS), lung, heart, placenta, mammary gland and gastrointestinal tract (GIT) [37, 38]. Apelin is an adipokine secreted and produced by white adipose tissue in mice and humans. It is also included in cardiovascular function. Apelin plays a role in the CNS and regulation of food intake and water balance. In the contrary, results of the other studies are confusing [39, 40].

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Apelin has a role in energy metabolism: as it improves sensitivity of insulin in insulin-resistant obese mice, and it is related to an increase in glucose uptake in skeletal muscle [41, 42]. Synthesis of apelin is affected by insulin and plasma apelin levels and it is increased in obesity in association with hyperinsulinaemia [43]. In our study, apelin had a significant correlation with glycosylated hemoglobin.

The relationship of apelin and diabetes in humans are still controversial. On the other hand, increased apelin level in obese subjects and type 2 diabetics were reported in some studies. Whereas other authors revealed decrease apelin in obese subjects with newly-diagnosed type 2 diabetes [44-48].

We found that a very limited study was done to assess apelin concentrations in type 1 diabetes. Two studies reported increase in apelin in type 1 diabetics, one of them was in children [49] and the other one was in adults [50]. On the other hand, a new study revealed that apelin levels is the same in diabetics and no diabetics [51]. In our study, we found that although apelin concentrations were increased, it had no relation to body mass index in diabetic patients. This means that obesity is not the main factor affecting apelin levels in diabetic patients which is in agreement with other studies [38, 44, 45, 52].

We conclude that apelin concentrations were increased in diabetic patients and it is affected by obesity. It is related to glycaemic balance and even insulin sensitivity. Diabetic patients had endothelial dysfunction and elevation of apelin, but they does not related to each other. NO is related to diabetic nephropathy and atherosclerosis.

Further large study is recommended to detect the relationship of apelin with vascular affection by assessing large number of diabetics with and without complication. Apelin is a beneficial adipokine and is a promising therapeutic target in metabolic disorders as it had anti diabetic properties.

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