Evaluation of Apelin 36 Level and Endothelial Function In Children with Chronic Kidney Disease

Azza Ali Gabr [2], Randa Kamal abdelRaouf[1], Safaa Taha Zaki[2], Hafez Mahmoud Bazaraa[4], Mona Hamed Ibrahim[3], Enas Abdel Rasheed[3], Mona Abd Elwahab[2]

[1] Medical Studies Department, Institute of Postgraduate Childhood Studies, Ain ShamsUniversity

[2]Child Health Department, [3]Clinical and Chemical Pathology Department, National Research Centre, Cairo, Egypt.

[4]Pediatric Nephrology Unit, Pediatrics Department, Kasr Al Aini Faculty of Medicine, Cairo University.

Abstract

Background: The life expectancy of patients with chronic kidney disease (CKD) is markedly reduced due to premature cardiovascular death. Endothelial dysfunction (ED) is closely linked to cardiovascular disease and subsequent morbidity and mortality in patients with chronic kidney disease. Also (CKD) in children affects their physical development as well as premature death that result from heart disease. Apelin is an adipocytokine that recently generated much interest; however, its role in (CKD) remains to be clarified.

Aim: Assessment of Apelin level and its correlation with markers of ED (V CAM-1), lipid profile and growth parameters in a sample of Egyptian children with chronic kidney disease.

Methodology: The present study was carried out in the pediatric hospital of Abou El Reash, Cairo University and the National Research Center (NRC), during the period between March 2011 to March 2013. The study included 66 clinically stable (CKD) patients and 20 healthy volunteers of the same age and sex. Serum levels of Apelin 36 and lipid profile; (high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, triglycrides) were measured. Endothelial function was evaluated by measuring vascular cell adhesion molecule (V cam-1) in serum.

Results: (V CAM-1) levels were significantly elevated in children with (CKD) as compared to controls. No change in Apelin level between patients and controls. Apelin was correlated positively with high density lipoprotein. (V CAM-1) level was positively correlated to triglycride, weight and height for age percentiles, and no correlation was found between apelin and (V CAM-1).

Conclusions: Serum Vcam-1 level was significantly higher in patients than controls and correlated positively with serum triglyceride level, also where highest in subjects having weight and height at or below the third percentile for age. There was no change in the level of Apelin between patients and controls and Apelin was positively correlated with (HDL) level and no significant correlations was found between Apelin and (V CAM-1) level. Independent from classical cardiovascular risk factors. Height and weight were found to be inversely associated with endothelial dysfunction in children with (CKD).

Keywords: Apelin, Endothelial function, Chronic Kidney Disease, and Children.

تقييم مستوى الابيلين والحالة الوظيفية لبطانة الاوعية الدموية فى الاطفال مرضى الاعتلال الكلوى المزمن

المقدمة: لقد ارتفعت معدلات الوفيات لمرضى الفشل الكلوى المزمن بشكل ملحوظ. وعلى الرغم من التحسن الكبير فى العلاج ببدائل الكلى، فان المرضى الذين يعانون من الداء الكلوى بمراحله الاخيره لديهم أعلى معدل للاصابة بامراض القلب عن غيرهم فى سن مماثلة. ويرتبط خلل الحالة الوظيفية لبطانة الاوعية الدموية بامراض القلب ومن ثم يرتبط بالحالة العامة لمرضى الاعتلال الكلوى المزمن. ويعد الابلين نوع من انواع الادبيوكاين المكتشفة حديثا ولا يزال دوره وعلاقته بمرضى الاعتلال الكلوى المزمن مجال للبحث والدراسة.

الهدف: الهدف من هذه الدراسة هو قياس مستوى الابلين فى الاطفال مرضى الاعتلال الكلوى المزمن وتقييم الحالة الوظيفية لبطانة الاوعية الدموية وكذلك القياسات الانثروبومترية ومستوى الدهون.

الاساليب: اشتملت هذه الدراسة على ٩٠ طفل منهم ٦٦ طفل مريض بالاعتلال الكلوى المزمن من الجنسين ويتراوح اعمارهم من (٣ إلى ١٨) سنة المترددين بصورة منتظمة على عيادة الكلى ووحدة الديلزة الدمويه بمستشفى الأطفال جامعة القاهرة.وتم تقسيمهم. ٢٠ اخرين من الاصحاء لهم نفس العمر والجنس من المترددين على العيادة الخارجية بمستشفى الاطفال جامعة القاهرة. تم قياس مستوى الابيلين ٣٦ وVcam-1 فى الدم وقياس مستوى الدهون وكذلك تم الكشف الاكلينيكى الكامل على الاطفال وقياس القياسات الانثروبومترية لهم.

النتائج: ارتفاع مستوى ال (V CAM-1)فى الاطفال المرضى عن الاصحاء بينما لايوجد فارق فى مستوى الابيلين فى المرضى عن الاصحاء. ولقد اوضحت هذه الدراسة وجود علاقة طردية بين مستوى الابيلين والبروتين الدهنى عالى الكثافة.ووجود علاقة طردية بين كل من (V CAM-1) والدهون الثلاثية وعكسية بين (V CAM-1) وكلا من الطول والوزن. وتوصلت الدراسة الي أن مرضى الاعتلال الكلوى المزمن يعانون من خلل فى الحالة الوظيفية لبطانة الاوعية الدموية والذى اوضحه ارتفاع مستوى ال (V CAM-1) فى الاطفال المرضى عن الاصحاء بينما لا يوجد علاقة بينه وبين مستوى الابيلين الذى لم يتغير فى المرضى عن الاصحاء. وان مستوى الابيلين ارتبط بعلاقة طردية بمستوى البروتين الدهنى عالى الكثافة وارتبط ال (V CAM-1) بمستوى الدهون الثلاثية.

Introduction:

The life expectancy of patients with chronic kidney disease (CKD) is markedly reduced due to premature cardiovascular death (Sarnak et al., 2003).

Despite considerable improvements in renal replacement therapy, patients with end stage renal disease (ESRD) have a higher prevalence of cardiovascular disease (CVD) than other populations of a comparable age (Amy et al., 2009).

The adipose tissue is now known to be a hormonally active organ that releases a large number of bioactive proteins regulating not only body weight and energy homeostasis, but also insulin resistance, blood lipids, endothelial health, coagulation, fibrinolysis and inflammation. Fat tissue secretes a number of hormones named adipocytokines; including leptin, adiponectin, apelin, resistin, vaspin, visfatin, as well as proinflammatory cytokines such as tumor necrosis factor α and interleukin- 6 (Guzik et al., 2006). Recent data suggest that these hormones also have immunomodulatory features and they are involved in inflammatory processes leading to atherogenesis (Sledzinska et al., 2009). Apelin is a newly discovered adipocytokine, produced by white adipose tissue (Jolanta et al., 2011), Its effect is through a cell surface G protein-coupled receptor called APJ, which has structural similarity with angiotensin type I receptor (Lee et al., 2000). Apelin is strongly expressed in the heart, and also in the large vessels, coronary vessels, endothelial cells, liver, kidney, adipose tissue, gastrointestinal tract, brain, adrenal glands (Boucher et al., 2005). Apart from acting on physiological cardiovascular regulation, Apelin may be involved in the pathophysiology of the cardiovascular system (Kleinz et al., 2005). Studies performed on mice knocked out for the apelin receptor gene have suggested the existence of a balance between angiotensin II signalling, which increases blood pressure and apelin signalling, which lowers blood pressure (Ishida et al., 2004). It has also been found to exhibit powerful inotropic activity producing its effects not only in normal hearts but also in failing hearts, raising the possibility that apelin might prove of value in the treatment of heart failure (Berry et al., 2004). In a recently published study, it was reported that apelin and its cognate G protein-coupled receptor APJ was widely represented in the heart and vasculature, and was emerging as an important regulator of cardiovascular homeostasis (Japp et al., 2008). Studies reported that apelin level decreased in hemodialyzed patients (El-Shehab et al., 2010). Moreover, the apelin-APJ pathway is thought to provide a mechanism for systemic endothelial monitoring of tissue perfusion and adaptive regulation of cardiovascular function (Sheikh et al., 2008). Detailed clinical investigation is now required to establish the role of apelin in human cardiovascular physiology and pathophysiology, and to determine the therapeutic potential of augmenting apelin signaling in patients with heart problems (Sahar et al., 2010).

To date, there are no data on possible relations between apelin and consequences of endothelial dysfunction. The Endothelium is the largest organ in the body strategically located between the wall of blood vessels and the blood stream. Vascular endothelium is a dynamic endocrine organ that regulates vascular tone, local homeostasis, and the fibroinflammatory proliferative process (Kensuke 2002). Endothelial cell damage or injury is invariably associated with such clinical conditions as thrombosis, hypertension, renal failure and atherosclerosis (Malyszko et al., 2008).

Endothelial "activation" is an injury response mechanism, with impaired endothelium dependent vasodilatation (EDV), increased adhesion of platelets and leukocytes, and is a putative first step in atherogenesis (Axelsson et al., 2007).

Aim:

The aim of this study was to investigate the serum Apelin and (V CAM-1) levels in children with chronic kidney disease, also to assess their relation to anthropometric parameters and lipid profile.

Patients And Methods:

The present study was carried out in the pediatric hospital of Abou El Reash, Cairo University and the National Research Center (NRC), during the period between March 2011 to March 2013. The study was performed on 90 children of both sexes, aged (3- 18) years, who were classified into 3 groups: Group (1) 27 patients with (CKD) stage 4 (estimated GFR< 30 ml/ min/ $1.7m^2$ by Schwartz formula (Schwartz et al., 1976) on conservative treatment. Group (2) 39 patients with End stage renal disease on regular hemodialysis for at least 6 month. Group (3) Twenty apparently healthy children of the same age and sex who were recruited from the outpatient Clinic of Abou ElReash Hospital, as controls. The patients met the following criteria: a stable clinical state, no thrombosis or inflammation and no complications as uncontrolled hypertension or acute heart failure. The hemodialysis group underwent regular hemodialysis for (3- 4) hours a day, three times a week using Fresenius Medical Care 4008 B machine with bicarbonate dialysate and biocompatible filter membrane (polysulofane).The prescription of each session was done according to the dry weight, weight gain between sessions, biochemical parameters and clinical condition of the patient.The causes of chronic kidney disease were chronic glomerulonephritis (n= 3), hereditary (n= 3), renal cystic disease (n= 7), urinary tract disorder (n= 21), metabolic (oxalosis) (n= 1), hemolytic uremic syndrome (n= 1), others (n= 11), undertermined (n= 20).

Each child (Patients& Controls) were subjected to full medical history, patients were subjected also for full medical history Including: age of onset, duration of the disease, treatment regimens for cases. Thorough clinical examination, with particular emphasis on measurement of vital signs and blood pressure, growth assessment through measurements of weight and height. Weight for age, height for age all were performed according to World Health Organization (WHO) growth curves.

Laboratory Investigations:

Six ml of peripheral venous fasting blood samples were withdrawn from every patient and control subject under complete aseptic conditions between 8.00 and 9.00 a.m.. Serum Apelin 36 levels were assayed by EIA kits from Phoenix Pharmacuticals. INC. Marker of endothelial cell injury Serum (V CAM-1) levels were assayed using IBL International GMBH ELISA Kit Cat. No:BE59051.Hamburg, Germany. Lipid profile (Cholesterol, Triglycride, HDL, LDL (Staubio Kit). Hemoglobin, hematocrit, platelet count, albumin concentration, were measured by standard laboratory methods. Dialysis adequacy using Kt/V: Fractional urea clearance(single pool kt/v), calculated from pre and post dialysis urea by natural logarithmic formula (Stuart et al., 2004), calculated for hemodialysis patients.

All subjects were informed about the aim of the study and gave their consent. The study was approved by the local Medical National Reasearch Center Ethic Committee.

Statistical Analysis:

The results were analyzed using SPSS version 16 as mean± SD. Comparisons of the mean differences in the studied parameters between

controls and (CKD) patients were performed using Anova test. Correlations between (V CAM-1), Apelin and other variables were evaluated by Pearson's correlation coefficient (r). The level of statistical significance (p< 0.05).

Results:

The patients mean age was 9.7± 3.7 for (CKD) patients on conservative treatment and 11.1± 2.8 for patients on regular hemodialysis.The main clinical and biochemical data of the patients with chronic kidney disease are presented in Table 1. There were no statistically significant differences between patients and controls regarding age, HDL, apelin levels. Height and weight were lower in (CKD) patients than those of the controls (p< 0.037), (p< 0.001) respectively. LDL, Cholesterol and triglyceride levels were higher in the control group than in patients (p< 0.001). Serum Apelin did not differ between the three studied groups. Serum (V CAM-1) levels were higher in (CKD) patients than in healthy controls (p< 0.001) Table 1. Age range of our patients was (3- 18) years, weight range (10- 61) kg and height range 70-151cm. It was found that 83.3% of patients had height below 3rd percentile and 65.15% of patients had weight below 3rd percentile. Tables 2, 3 Patients had significantly lower values regarding weight, height, Weight for age (percentiles) height for age (percentiles) than controls (P< 0.000, P< 0.002) respectively. Tables 4, 5 show that (V CAM-1) levels were found to be higher in patients who had weight and height below 3rd percentile (p< 0.012, p< 0.007) respectively, also Apelin levels were found to be lower in those patients who had weight and height below 3rd percentile but without significant difference (p< 0.675, p< 0.350) respectively. Table 6. There was a significant positive correlation between, Apelin and HDL (r= 0.399, p< 0.000) and a negative correlation with LDL/ HDL (r= -0.237, p< 0.032) Table 6 and figure (1), there was a significant positive correlation between (V cam-1) and triglycride (r= 0.259, p< 0.019).

Table (1) Clinical and biochemical data of patients with chronic kidney disease and controls

Parameters	(CKD) patients on Conservative treatment (n= 27)	(CKD) Patients On Hemodialysis (N= 39)	Controls (N= 20)
	Mean±SD	Mean±SD	Mean±SD
Age (Years)	9.7 ± 3.7	11.1 ± 2.8	10.5 ± 3.3
Sex (M/F)	15/12	26/13	8/12
Height (Cm)	114.9±23.8	120.5±15.5b	129.5±17.6
Weight (Kg)	25.33±11.5	23.8±6.4a	34.9±13.9
SBP (Mmhg)	88.9±10.9	116.7±13.6a	102.5±10.2
DBP (Mmhg)	60.7±6.2	76.8±9.5a	61.5±6.7
LDL (mg/dl)	51±31.6	64.4±24.1a	92.6±27.6
HDL (mg/dl)	53.1±10.2	51.2±14.7	45±6.5
Triglycride (mg/dl)	112.5±38.2	133.3±40a	155.7±27
Cholesterol (mg/dl)	72.3±30.9	107.8±52.5b	98.5±33.3
Apelin 36 (ng/ml)	5.3±2.2	4.4±2.9	4.9±1.3
(V CAM-1) (ng/ml)	1632±903.4	2198.8±1019.8a	1239.2±297.3

Data are expressed as mean±SD. (CKD), chronic kidney disease; HDL, high density lipoprotein; LDL, low density lipoprotein; VCAM-1, vascular cell adhesion molecule.SBP: Systolic blood pressure, DBP:Diastolyic blood pressure. a Significant at p < 0.001, b Significant at p < 0.01

Table (2) Classification of the studied groups according to their Weight percentile

Weight Percentile	Conservative (N)	Hemodialysis (N)	Control (N)	P Value
1 (≤3rd Percentile)	16	34	1	0.000
2 (>3rd, ≤ 25 percentile)	7	5	8	
3 (>25 Percentile)	4	0	11	

Chi-Square test Significant at p < 0.05, (n) number of subject

Table (3) Classsification of the studied groups according to their Height percentile

Height Percentile	Conservative (N)	Hemodialysis (N)	Control (N)	P Value
1 (≤3rd Percentile)	20	31	6	0.002
2(>3rd, ≤ 25 percentile)	5	8	11	
3 (>25 Percentile)	2	0	3	

Chi-Square test Significant at p < 0.05, (n) number of subject

Table (4) Apelin in relation to Weight, and Height percentiles in (CKD) patients

		Apelin Level	P Value
Weight Percentile	≤ 3rd Percentile	4.58	0.675
	>3rd, ≤25 Percentile	5.08	
	>25 Percentile	5.07	
Height Percentile	≤ 3rd Percentile	4.5	0.350
	>3rd, ≤25 Percentile	5.1	
	>25 Percentile	6.1	

ANOVA test P value is significant at the0.05

Table (5) (V CAM-1) in relation to Weight, and Height percentiles in (CKD) patients:

		(V CAM-1) Level	P Value
Weight Percentile	≤ 3rd Percentile	2038.1	0.012
	>3rd, ≤25 Percentile	1636.05	
	>25 Percentile	1240	
Height Percentile	≤ 3rd Percentile	2036.6	0.007
	>3rd, ≤25 Percentile	1426.4	
	>25 Percentile	1035.5	

ANOVA test P value is significant at the0.05

Table (6)Correlation between Apelin and (V CAM-1) and Lipid profile

	Apelin		(V CAM-1)	
TRIG	R = -0.007	P= 0.949	r = 0.259*	P= 0.019
CHOL	R= -0.060	P = 0.653	R= -0.096	P= 0.475
HDL	r = 0.399**	P= 0.000	R= -0.042	P= 0.711
LDL	R= -0.190	P= 0.088	R= -0.005	P= 0.963
Ldl/Hdl	r= -0.237*	P = 0.032	R= 0.016	P = 0.886

*Correlation is significant < 0.05 level (2-tailed).
**Correlation is significant < 0.01 level (2-tailed).

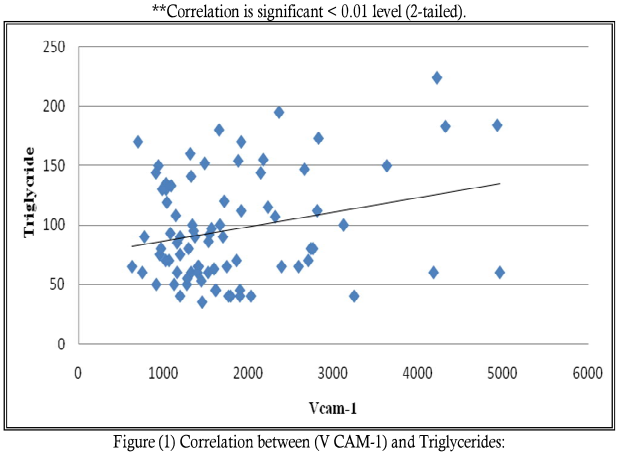

Figure (1) Correlation between (V CAM-1) and Triglycerides:
Correlation between (V CAM-1) and Triglycerides r= 0.259, p= 0.019

Discussion:

This study has investigated the possible correlations between the newly identified adipocytokine; Apelin and markers of ED, in a sample of Egyptian children with (CKD) and demonstrated that serum levels of apelin were not changed between the patients and controls this finding agreed with Sheikh et al. (2008) who demonstrated that apelin and APJ were upregulated in the heart and skeletal muscle following myocardial injury and suggested that apelin expression remained restricted to the endothelium. Experiments with cultured endothelial cells in vitro showed apelin mRNA and protein levels to be increased by hypoxia, through a hypoxia-inducible factor-mediated pathway. Since patients on renal replacement therapy suffered from hypoxia (due to anemia and other factors) (Sheikh et al., 2008). Not all adipokines promote

endothelial dysfunction and/or thrombosis. Adiponectin is considered to be protective against thrombosis and atherosclerosis (Ekmekci et al., 2006).

Serum levels of (V CAM-1) were found to be higher in (CKD) patients than in controls which agreed with Attalah et al. (2011) who found that levels of (V CAM-1) and ICAM-1 were significantly higher in of patients with (CKD). In our study (V CAM-1) level was positively correlated with triglycride level which agreed with, Bartus et al. (2005) who found that hypercholesterolemia and hypertriglyceridemia induce endothelial dysfunction and therefore lead to atherosclerosis. Through impairment of NO-dependent vasodilation. Also agreed with Diana et al. (2008) who found association of triglycerides with sICAM-1 may indicate a particular impact of lipid metabolism on endothelial reaction. We found that Apelin level was positively correlated with HDL level which represent a strong, and coherent cardiovascular risk marker seen across all populations, with higher levels of HDL cholesterol being associated with decreased incidence of coronary artery disease. The cardiovascular protective effects of HDL particles are attributed, in great part, to the ability of HDL particles to promote cellular cholesterol efflux from lipid-laden macrophages within the atherosclerotic plaque. HDL also has pleiotropic effects that protect the vascular wall, at least in vitro. These effects include potent anti-inflammatory and antioxidant properties and the modulation of vascular endothelial function. The mechanisms by which HDL exert their function on the vascular endothelium is dependent on HDL particle size, protein (proteome) and lipid (lipidome). preventing uncoupling of NADPH oxidation and nitric oxide synthesis and increasing endothelial nitric oxide synthetase abundance. Furthermore, HDL can modulate the activation of NF-κB and the expression of cell adhesion molecules, an early step in endothelial dysfunction (Campbell S. et al., 2013) (V CAM-1) level was found to be highest in children below the third percentile for weight and height. Children with (CKD) suffer from growth impairment which may be due to malnutrition, hypoalbuminemia or disruption of the hypothalamic-pituitary growth hormone axis that contributes to the growth hormone-resistant state in uremia. Growth failure in those children is considered to be an additional cardiovascular risk factors and endothelial dysfunction in children with (CKD). Our data agreed with Lilien et al. (2004) who support the theory that a disturbance in the GH-IGF axis contributes to the endothelial dysfunction of renal failure. Treatment with rhGH not only improves growth but may also favorably influence the risk for atherogenesis.

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