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Study of Neuropeptide Y and its Relation to the Cardiovascular Complications in End Stage Renal Disease

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Abstract: Chronic renal insufficiency is a situation characterized by high plasma concentration of neuropeptide Y (NPY) which is known to interfere with cardiovascular function; so it is possible that it is involved in the high cardiovascular related morbidity. Neuropeptide Y is a vasoactive neuropeptide widely distributed in the central and peripheral nervous system. The metabolism of NPY in patients with renal failure seems to be altered as the enzyme peptidase activity which degrades this neuromediator is altered in renal failure. NPY was measured in several studies in patients with end stage renal disease and found to be mildly to markedly elevated. Also NPY was reported to be related to arterial blood pressure, fluid overload, heart rate, left ventricular function and structure. The present study was designed to estimate plasma NPY in patients with end stage renal disease (ESRD) to clarify the relation between its plasma level and the cardiovascular system complications specially the effect on blood pressure, heart rate and the development of structural and functional change of the left ventricle. The study included 51 CRF patients on regular hemodialysis, 20 patients with renal impairment on conservative treatment in addition to 20 healthy individuals, matched for age and sex, as a control group. All patients and control subjects were subjected to full medical history, thorough clinical examination, estimation of serum urea, creatinine, calcium and phosphorous, cholesterol and triglycerides (standard methods), ECG and echocardiography and quantitation of serum C-reactive protein by immunoturbidimetric assay and plasma NPY level using competitive ELISA kit. A significant increase was found in the level of plasma NPY in the hemodialysis and renal impairment patients versus the control group and in the hemodialysis group compared to the renal impairment group. An inverse correlation was recorded between plasma NPY and ejection fraction in both the renal impairment group and the hemodialysis group and a direct correlation between plasma NPY level and left ventricular mass in the renal impairment group and in the hemodialysis group. It can be concluded that plasma NPY is elevated in patients with renal impairment treated conservatively and more so in patients with CRF on hemodialysis. Plasma NPY is considered a nontraditional risk factor for stratification of cardiovascular complications emerging in ESRD, especially owing to its effect on heart rate and on left ventricular structure and function. Echocardiography and plasma NPY are non invasive cost effective tools for screening and early detection of cardiovascular alterations complicating ESRD.

Key words: Neuropeptide Y · Cardiovascular Disease · End Stage Renal Disease · C reactive protein

INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of mortality and morbidity in the general population worldwide. CVD risk is more pronounced in patients with end stage renal disease (ESRD); it accounts for death in more than 50% of patients undergoing regular dialysis. Moreover, the risk for CVD in a young patient

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with ESRD is similar to that of an elderly subject from the non renal population [1]. It is believed that all dialysis patients should be considered as very high cardiovascular risk individuals and that aggressive prevention measures should be applied to reduce morbidity and mortality [2]. In uremic patients a marked increase in left ventricular mass is seen very early in the course of renal failure. Left ventricular disease is already present in 85% of patients starting dialysis.Sixteen percent of patients had systolic dysfunction, 41% concentric LV hypertrophy, 28% LV dilatation and 16% had normal cardiac findings on echocardiography[3].

Traditional risk factors for CVD such as age, sex, hypertension, diabetes and dyslipidemia cannot explain the excess mortality and morbidity in renal patients [4]. This amplification in CVD risk in renal patients has been attributed to several emerging risk factors such as oxidant stress, hyperhomocysteinemia, hyperphosphatemia, chronic low grade inflammation and arteriosclerosis [5].

ESRD is now considered a prototypical situation of chronic inflammatory state [6] and C-reactive protein (CRP), a non specific marker of inflammation, is reported as a fundamental biomarker for cardiovascular risk stratification in these patients [7]. There is consistent evidence that CRP and proinflammatory cytokines such as IL-1beta, IL-6 and TNF-α are risk factors for atherosclerotic complications and predict death and adverse cardiovascular outcomes in these patients [8].

The heart and kidney are inextricably linked in terms of hemodynamic and regulatory functions. Communication between these two organs occurs at multiple levels including the sympathetic nervous system [9]. Increased sympathetic outflow may occur in a number of diseases, either as a primary event contributing to development of the disease or as secondary to the underlying disease [10].

Plasma norepinephrine (NE) in ESRD patients was found to be high in most studies; chronic renal failure was considered a situation characterized by enhanced sympathetic activity. The interpretation of plasma NE as a marker of sympathetic activity is complex in patients with renal failure because circulating NE represents only a small proportion of the neurotransmitter amount secreted from adrenergic nerve terminals and because these patients display metabolic alterations which may alter the plasma concentration of this substance [11].

Neuropeptides are contained in and released from a wide range of nerves. Chemically distinct, they exhibit characteristic patterns with a wide range of diverse biological activities. The concept of cotransmission within the autonomic nervous system was first suggested by Burnstock [12], through this study of ATP in sympathetic nerves, followed by the realization that neuropeptides are also contained in both sympathetic and cholinergic nerves.

Neuropeptide Y (NPY) is a vasoactive neuropeptide widely distributed in the central and peripheral nervous system and response in target tissues results from interaction of the neuropeptide with specific G-protein coupled receptors [13]. It is well demonstrated that nerves in the gut are an important source of circulating neuropeptide Y in humans. Neuropeptide Y has a biphasic disappearance from plasma and the corresponding half-lives are 4-6 min and 20-40 min [6].

Increased plasma neuropeptide Y(NPY) levels have been observed in situations characterized by high sympathetic activity such as physical exercise, heart failure and cardiac ischemia. Furthermore, plasma NPY is increased in over one half of patients with pheochromocytoma [14].

Neuropeptide Y has been reported to be consistently elevated in patients with end stage renal disease, a phenomenon which may depend on the fact that the enzyme (peptidase) activities that degrade this neuromediator are altered in renal failure [6, 14]. In a study done by Sucajtys-Szulc *et al.* [15], it was found that the NPY gene expression in the hypothalamus of CRF rats was significantly higher than in the hypothalamus of control rats. Moreover, it was found that serum NPY concentration in CRF rats was higher than in control rats.

Neuropeptide Y is coreleased with NE during sympathetic nerve stimulation and is extensively involved in cardiovascular (CV) regulation because it may modulate heart rate, blood pressure, cardiac excitability, ventricular function as well as coronary blood flow. NPY behaves as a stress hormone because its plasma concentration is markedly increased in septic shock, in myocardial infarction and it predicts survival in patients admitted to coronary care units with or without myocardial infarction [11, 16].

NPY is present in myocardial tissue, in the sympathetic innervation of all parts of the conduction

system and also in nerve fibres in the heart that do not represent sympathetic fibres. NPY receptor stimulation enhances DNA synthesis in cardiomyocytes and directly stimulates hypertrophy. Furthermore, it has been recently demonstrated that the hypertrophic effect of NE via α-adrenoreceptors stimulation can be modulated by corelease of NPY from ventricular intramural nerve endings [17].

High plasma NPY was associated with systolic dysfunction and this link was independent of and stronger than that of plasma NE. Also this association was evident across a wide range of ejection fraction values extended into the normal and high range. In isolated cardiomyocytes NPY alone has a positive inotropic effect, while it abolishes the contractile response induced by β-adrenergic stimulation [18].

Few studies showed that NPY elicits positive and negative contractile effects in cardiomyocytes through Y1 and Y2 receptors, respectively [19]. In addition the endocardial endothelial cells play an important role in regulation of cardiac function by releasing cardioactive factors such as endothelin-1, angiotensin II, nitric oxide and NPY which may play a role in increasing intracellular free Ca and the frequency of beating of cardiomyocytes [20].

In humans, independently of ventricular function, the concentration of NPY and NE at myocardial level decreases in parallel after sustained stimulation by dopamine and NPY and NE stores are depleted in the failing human myocardium. Conversely, the plasma concentration of NPY and NE is markedly increased in patients with heart failure and such increase is proportional to the severity of the disease indicating that these neurohormones participate in the counter-regulatory response to left ventricular failure [11].

Because CV risk in patients with chronic renal diseases is much increased, it is possible that the vasoactive neuromediator NPY, which is extensively involved in cardiovascular regulation, is implicated in the high CV morbidity and mortality of these patients.

This study was designed to assess plasma level of neuropeptide Y (NPY) in patients with end stage renal disease (ESRD) on hemodialysis and on conservative treatment in conjunction with echocardiographic assessment as well as determination of C reactive protein (CRP), the well established cardiovascular risk factor in ESRD, in order to clarify the link between NPY and cardiovascular complications in patients with ESRD.

PATIENTS AND METHODS

Ninety-one subjects were enrolled in this study. They were divided according to data from initial clinical and routine laboratory evaluation into the following three groups:

Group A: This included 51 uremic patients on regular hemodialysis 3 times per week in 4 hours sessions in Theodor Bilharz Research Institute. Thirty- one were males and 20 were females with ages ranging between 34-76 years with a mean of 57.1± 9.39.

Group B: This included 20 patients with renal impairment under conservative treatment (8 males and 12 females, age range between 30-73 years with a mean of 54.75±16.34.

The etiology of renal failure was variable among the 2 studied patient groups (hypertension, diabetes mellitus, urological causes and unknown causes). They were under antihypertensive treatment by beta blocker, ACEI, methyl dopa and alpha blocker.

Group C: This included 20 healthy control subjects (14 males and 6 females, age range between 32-70 years with a mean of 51.7±9.39). They were not hypertensive or diabetic with normal kidney function and normal echocardiographic findings.

All patients and control subjects were subjected to the following investigations:

1-Full Medical History: causes of renal failure, type of therapy received, duration of dialysis, past history related to cardio-vascular affection e.g. previous anginal episodes, thrombotic events, ECG documented arrhythmia etc.….

2-Thorough Clinical Examination: general as well as abdominal, cardiac and chest examination.

•Blood Sampling Technique: Following an overnight fast for at least 12 hours, 12ml venous blood was withdrawn by clean venipuncture from the antecubital vein: 2ml on EDTA for blood picture; 7ml without anticoagulation to be clotted and centrifuged. Separated serum was used for assessment of kidney functions, Ca, P and lipid profile on the collection day and aliquots of serum were stored at

-20°C for further estimation of CRP; 3ml on EDTA (for anticoagulation) and aprotinin (0.6TIU/ml of blood) to inhibit the activity of proteinases to be centrifuged at 1,600 x g for 15 min at 4°C for plasma separation to be followed by peptide extraction and storage at -20°C for further determination of plasma NPY.

3- Routine Laboratory Investigations: included CBC and quantitation of serum urea, creatinine, calcium and phosphorous, triglycerides, total cholesterol, HDL-and LDL- cholesterol by standard methods.

4- Electrocardiogram

5-Echocardiography: Standard transthoracic M. mode, two dimensional, continuous and pulsed wave Doppler echocardiograms were obtained soon after a session of routine hemodialysis using 2.5 MHZ transducer.

Left Ventricular Mass (LVM): was calculated according to the following formula:

$$LVM\ (g) = 1.04\ [(LVEDD + LVPWT + IVST)^3 - (LVEDD)^3] - 13.6g \quad [21]$$

LVEDD= left ventricle end diastolic dimension,
LVPWT= left ventricle posterior wall thickness,
IVST= interventricular septum thickness.

Ejection fraction (EF) which is the percentage change in LV volume between systole and diastole:

$$EF = \frac{LVEDV - LVESV}{LVEDV} \times 100$$

LVEDV= left ventricle end diastolic volume,
LVESV= left ventricle end systolic volume.

6- Special Laboratory Investigations:

1-Serum CRP Determination: using immunoturbidimetric assay (Randox laboratories, Ltd). Kone progress/ specific. In this assay system, sample is reacted with a buffer and anti-CRP coated latex. The formation of the antibody-antigen complex during the reaction results in an increase in turbidity, the extent of which is measured as the amount of light absorbed at 550nm. By constructing a standard curve from the absorbance of the standards, CRP concentration of sample can be determined.

2- Quantitative Determination of Plasma NPY: using the competitive ELISA kit from Phoenix Pharmaceuticals, INC, 330 Beach Road Burlingame, Ca 94010, USA with a minimum detectable concentration equal to 0.14 ng/ml. The immunoassay was preceded by peptide extraction procedure using a C 18- Sep column (Code RK SEPCOL-1) and 2 chromatographic solvents: Buffer A (RK-BA-1) and Buffer B (RK-BB-1).The sample was then freezedried using a lyophilizer and stored at -20°C until assayed. In the immunoassay the 2^{ry} antibody precoating the immunoplate binds to the Fc fragment of the 1^{ry} antibody whose Fab fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in samples. The biotinylated peptide interacts with streptavidin-horseradish peroxidase (SA-HRP) which catalyzes the substrate solution. The intensity of the yellow colour is inversely proportional to the quantity of peptide in standards and samples due to competitive binding of biotinylated peptide with standard or sample peptide to peptide antibody (1^{ry} antibody).The unknown sample concentrations can be obtained by extrapolation to the constructed standard curve.

Statistical Tools: Results were expressed as mean values ± standard deviation (SD) or number (%). Comparison between the mean values of each two groups was done using unpaired Student t-test. Comparison between categorical data [n(%)] was brought about by Chi square test. Correlation between parameters was performed using Spearman's rank correlation coefficient. SPSS Computer Program (Version 14 Windows) was used for data analysis. P value equal to or less than 0.05 was considered the threshold for significance.

RESULTS

This study was conducted on ninety one persons. They were divided into three main groups:

Group A: included 51 patients with CRF and on regular hemodialysis
Group B: included 20 patients with renal impairment on conservative treatment without hemodialysis.
Group C: included 20 apparently healthy individuals.

Results were summarized as mean values±SD or n (%) and were depicted in the following Tables (1-5) and Figures (1-4).

Table 1: Comparison of demographic and clinical features of the studied groups [Mean values±SD or n(%)]

				P value		
	Group (A) n=51	Group(B) n=20	Group (C) n=20	A vs C	B vs C	Avs B
Age (years)	57.1±9.39	54.75±16.34	51.7±9.39	NS	NS	NS
Male [n(%)]	31 (60.8%)	8 (40%)	14 (70%)			
Female [n(%)]	20 (39.2%)	12 (60%)	6 (30%)	NS	NS	NS
BMI *(kg/m²)*	25.73±4.54	26.3±3.65	27.49±4.0	NS	NS	NS
DOD (years)	6.31±4.03	--	--	--	---	--
SBP (mmHg)	134.3±21.67	139.0±21.25	118.25±8.16	P < 0.01 *	P<.001 *	NS
DBP(mmHg)	84.3±12.62	87.5±12.51	78.75±4.25	NS	P<.05 *	NS
Pulse (Beats/min)	85.61±9.38	84.85±13.26	79.2±10.2	P< 0.05 *	NS	NS

BMI=body mass index, DOD: duration of dialysis, n= number of patients, NS= Non significant, * = significant

Table 2: Comparison of renal functions, Ca, P and lipid profile of the studied groups (Mean values±SD)

				P value		
	Group(A) n=51	Group(B) n=20	Group (C) n=20	A vs C	B vs C	A vs B
S.creatinine *(mg/dl)*	7.9±2.07	3.7±2.07	1.22±0.17	P< 0.001*	P<0.001*	P<0.001*
S.urea *(mg/dl)*	138.8±27.5	125.6±47.39	30.4±4.33	P <0.001*	P<0.001*	NS
S. calcium *(mg/dl)*	9.0±1.17	9.0±1.12	9.6±0.7	P <0.05*	P <0.05*	NS
S. phosphorus *(mg/dl)*	5.2±1.76	5.4±1.3	3.33±0.84	P <0.001*	P<0.001*	NS
S. triglycerides *(mg/dl)*	230.9±103.5	202.2±109.6	153.15±13.8	P <0.01*	NS	NS
S. total cholesterol *(mg/dl)*	178.1±58.42	181.1±67.45	192.5±15.4	NS	NS	NS
S. HDL- cholesterol *(mg/dl)*	33.6±19.38	34.6±11.7	51.1±15.96	P <0.001*	P< 0.01*	NS
S. LDL- cholesterol *(mg/dl)*	100.4±55.7	106.1±58.3	110.77±28.2	NS	NS	NS

S.= serum, NS= nonsignificant, * = significant

Table 3: Comparison of the echocardiographic data in the studied groups [Mean values±SD or n(%)]

				P value		
	Group(A) n=51	Group(B) n=20	Group (C) n=20	A vs C	B vs C	A vs B
LVEDD *(mm)*	54.2±8.8	55.3±6.7	51.5±7.2	NS	NS	NS
LVESD *(mm)*	35.8±9.3	36.9±9.1	31.2±5.4	P<0.05 *	P<0.05*	NS
FS (%)	34.9±8.86	34.5±5.6	39.4±5.8	P<0.05 *	P<0.05*	NS
PWT *(mm)*	11.1±2.5	10.8±3.1	8.8±1.3	P<0.001*	P<0.05*	NS
IVST*(mm)*	11.4±2.6	9.9±2.5	8.8±1.2	P<0.001*	NS	P<0.05*
EF (%)	62.1±11.5	58.8±14.8	69.1±7.4	P<0.05 *	P<0.01*	NS
LVM (g)	297.2±110.9	277.1±118.6	188.7±45.1	P<0.001*	P<0.01*	NS
WMA [n(%)]	17(33.3 %)	3 (15 %)	0 (0%)	P<0.01 *	NS	NS
Diastolic Dysfunction [n(%)]	40 (78.4.%)	12 (60%)	0 (0%)	P<0.001 *	P<0.01*	NS
P. Effusion [n(%)]	5 (9.8%)	2(10%)	0 (0%)	NS	NS	NS
Pulmonary hypertension [n(%)]	10(19.60%)	7(35%)	0(0%)	NS	P<0.05*	NS
Calcification [n(%)]	21(41.20%)	3(15%)	0(0%)	P <0.01 *	NS	P<0.05*

LVEDD= left ventricle end diastolic dimension, LVESD=left ventricle end systolic dimension,
FS= fractioning shortening, PWT= posterior wall thickness, IVST= Interventricular septum thickness, EF= Ejection fraction, LVM=left ventricular mass, WMA=Wall motion abnormality, P. effusion= pericardial effusion, n= number of patients, NS= nonsignificant, * = significant.

Table 4: Comparison of serum CRP (mg/l) and plasma NPY (ng/ml) levels in studied groups (Mean values + SD)

				P value		
	Group(A) n=51	Group(B) n=20	Group (C) n=20	A vs C	B vs C	A vs B
CRP(mg/l)	7.67±2.61	4.88±0.94	1.86±0.44	P<0.001*	P<0.001*	P<0.001*
NPY(ng/ml)	14.27±4.0	11.38±2.92	3.66±1.76	P<0.001*	P<0.001*	P<0.01*

* = significant

Table 5: Correlation matrix between plasma NPY and some other parameters in patient groups

	Group(A) NPY (ng/ml)		Group (B) NPY (ng/ml)	
	r	P value	r	P value
Pulse (beats/min)	0.441	0.001*	0.575	0.008*
EF (%)	-0.350	0.013*	-0.519	0.019*
PWT (mm)	0.429	0.002*	0.571	0.009*
IVST (mm)	0.397	0.004*	0.505	0.023*
LVM (g)	0.462	0.001*	0.493	0.027*
CRP (mg/l)	0.210	0.373	0.097	0.498

r = correlation coefficient, * = significant

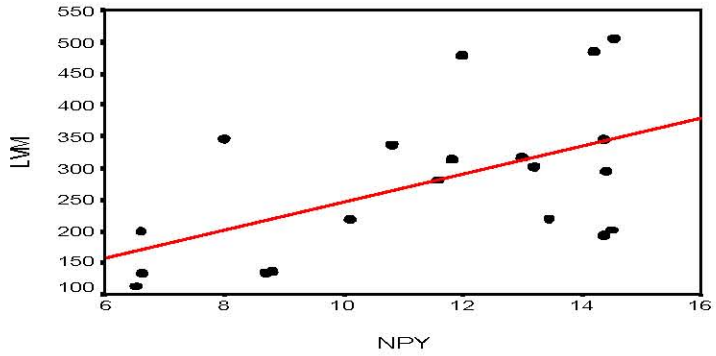

Fig. 1: Correlation between plasma NPY (ng/ml) and ejection fraction (%) in renal impairment patients *(r= -0.519; p= 0.019*)*, * = significant

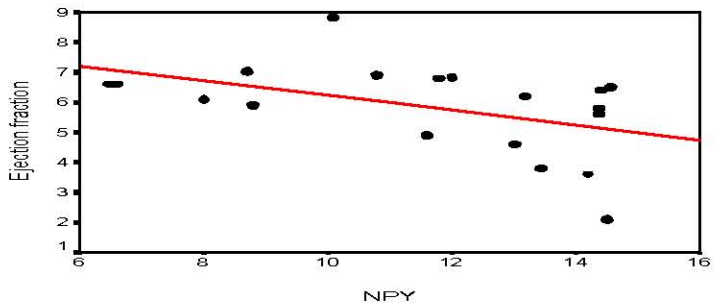

Fig. 2: Correlation between plasma NPY (ng/ml) and LVM (g) in renal impairment patients *(r= 0.493; p= 0.027*)*, * = significant

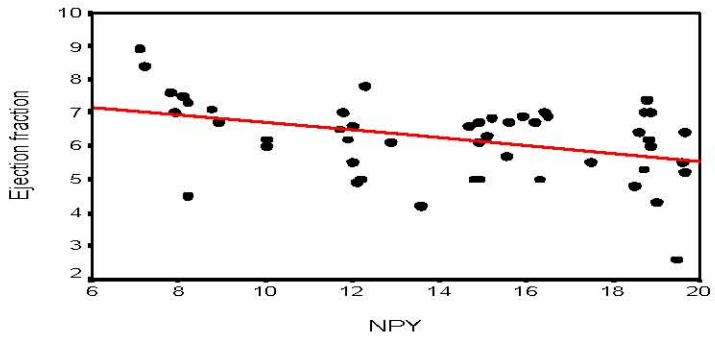

Fig. 3: Correlation between plasma NPY (ng/ml) and ejection fraction (%) in HD patients *(r=-0.350; p=0.013*)* , * = significant

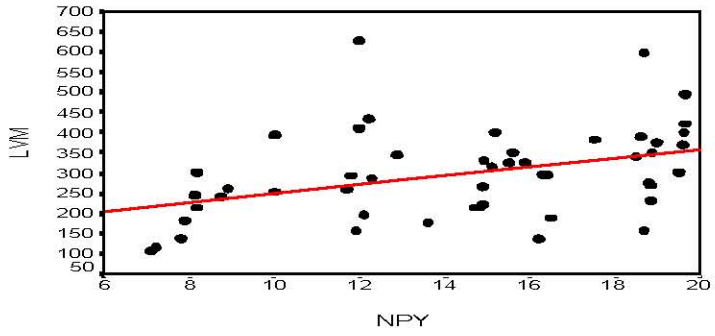

Fig. 4: Correlation between plasma NPY (ng/ml) and LVM (g) in HD patients (r= 0.462; p= 0.001 *), * = significant

DISCUSSION

Cardiovascular complications are the leading cause of mortality in patients with ESRD. Complications include coronary artery disease, left ventricular hypertrophy, heart failure and arrhythmia. Although traditional risk factors such as diabetes mellitus, hypertension and dyslipidemia are prevalent in ESRD, they are not sufficient to account for the high prevalence of cardiovascular mortality; thus the search for other non traditional risk factors that may be involved in pathogenesis of uremia is under intense study [22].

Increased plasma levels of the vasoactive neuropeptide Y (NPY) have been observed in situations characterized by high sympathetic activity as it behaves as a stress hormone [6]. Its plasma concentration is markedly increased in septic shock, in myocardial infarction and it predicts survival in patients admitted to coronary care units with or without myocardial infarction [16]. NPY is coreleased with NE during sympathetic nerve stimulation. It may modulate heart rate, blood pressure, cardiac excitability, ventricular function as well as coronary blood flow [11].

Studies demonstrated that NPY is elevated in patients with ESRD; a phenomenon which may depend on the fact that the activity of the enzyme (peptidase) that degrades this neuromediator is altered in renal failure [6]. NPY is extensively involved in cardiovascular regulation; it is possible that it is involved in the high cardiovascular morbidity in uremic patients. It causes prolonged vasoconstriction and vascular remodeling. It has pro-atherogenic action and in addition it exerts an effect on cell growth and hypertrophy [23].

The current study revealed a significant increase in systolic blood pressure in patients with renal impairment ($P<0.001$) and patients on hemodialysis ($P<0.01$) versus the control group. Moreover, a significant increase in diastolic blood pressure was found in renal impairment group versus control group ($P<0.05$), but there was no significant difference in diastolic blood pressure between hemodialysis and control groups. However, in agreement with these results Foley and Agarwal [24] stated that the pathogenesis of hypertension in renal failure is complex and arises from the interaction of hemodynamic and neuroendocrine factors.

No significant difference in serum total and LDL cholesterol was recorded between the different studied groups. However, there was a significant increase in triglyceride level in patients on hemodialysis versus the control group ($P<0.01$) and a significant decline in HDL cholesterol level in renal impairment ($P<0.01$) and hemodialysis ($P<0.001$) groups versus the control group. In harmony with the present study Krane and Wanner [25] reported that chronic kidney disease (CKD) is associated with a highly atherogenic lipid profile, characterized by elevated triglycerides and low HDL cholesterol.

The current study found a significant elevation in serum C-reactive protein (CRP) in the renal impairment and hemodialysis groups versus the control group ($P<0.001$ for both) and a significant increase in CRP in the hemodialysis group versus the renal impairment group ($P<0.001$). Current findings coincided with those of Quereshi *et al.* [26] and El-Shamy *et al.* [27] who found CRP to be markedly elevated in CRF patients, especially those on hemodialysis. Furthermore, Jeznach-Steinhagen *et al.* [28] reported that there is evidence that patients with chronic kidney disease (CKD) are in a state of chronic inflammation with activation of C-reactive protein and proinflammatory cytokines and is associated with increased oxidative stress and endothelial dysfunction. There is consistent evidence that CRP and proinflammatory cytokines such as IL-1beta, IL-6 and TNF-α are risk factors for atherosclerotic complications and predict death and adverse cardiovascular outcomes in these patients [8]. Increasing evidence suggests that CRP may be directly involved in atherothrombogenesis: CRP is present in the vessel wall, where it induces expression of the adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) by endothelial cells and serves as a chemoattractant for monocytes. CRP opsonizes LDL and facilitates native LDL entry into macrophages. Also it binds to plasma membranes of damaged cells and activates complement which seems to be crucial for mutation of atherosclerotic lesions [29].

Regarding echocardiographic findings, the current study detected a significant increase in left ventricular end systolic diameter in the renal impairment and hemodialysis groups versus the control group ($P<0.05$ for both). Furthermore, there was a significant decrease in fractioning shortening (FS %) in the renal impairment and hemodialysis groups versus the control group ($P<0.05$ for both); and a significant reduction in ejection fraction in the renal impairment group ($P < 0.01$) and the hemodialysis group ($P < 0.05$) versus the control group. Recent guidelines and position statements have therefore defined chronic kidney disease (CKD) as a cardiovascular risk equivalent and patients in all stages of CKD are considered in the highest risk group for development of cardiovascular disease (CVD). Moreover, studies showed that heart failure is the main

cardiovascular complication that occurs in renal patients and its incidence increases proportionally with the reduction of glomerular filtration rate [30].Also Lisowska and Musial [31] stated that heart failure is highly prevalent in ESRD patients; upon starting dialysis, 37% of patients had a previous episode of heart failure, doubling the risk of death, both systolic and/or diastolic function may be impaired and 15% of patients on dialysis therapy may have systolic dysfunction.

The present study found a significant increase in left ventricular mass in the renal impairment group (P<0.01) and the hemodialysis group (P<0.001) compared to the control group. In agreement with these results, Kimura *et al.* [32] stated that left ventricular hypertrophy (LVH), which is a strong predictor of mortality in patients with ESRD, is present in over 70% of patients commencing dialysis. In fact, pressure and volume overload, that are inherent to the abnormalities of homeostasis typical of CKD, lead to concentric/eccentric LVH. Initially, LVH is adaptive because energy is spared by maintaining stable wall stress. However, in the long term, LVH becomes maladaptive, inducing systolic and/or diastolic dysfunction that, in turn, leads to symptomatic left ventricular failure. Increased left ventricular mass is associated with severe renal dysfunction and a higher cardiovascular mortality [30]. Also mild to moderate renal impairment is associated with an increase in LVM which is associated with higher mortality [33].

Study findings revealed that the level of plasma NPY exhibited a significant elevation in both the renal impairment group (P<0.001) and the hemodialysis group (P<0.001) versus the control group and a significant increase in hemodialysis patients as compared to renal impairment patients (P<0.01); findings which are in harmony with the results of Zoccali [11] who stated that NPY is elevated in patients with renal failure because its metabolism is impaired, as the activity of the enzyme (peptidase) that degrades this neuromediator is altered in renal failure. Furthermore, it is known that sympathetic over activity is an established trigger of cardiovascular morbidity; ESRD is associated with high norepinephrine level and a high sympathetic output, thus because NPY is coreleased with NE, the link between this peptide and CV morbidity is well established [6]. Secretion of NPY may be enhanced by poor state of nourishment and stress induced by fluid volume overload in patients on HD and plasma NPY is removed by high flux dialyzer [34]. Also, NPY level correlated inversely with renal plasma flow and glomerular filtration rate and directly with norepinephrine in patients with hepatorenal syndrome [35].

In the present study, an inverse correlation was found between plasma NPY and ejection fraction in both the renal impairment group (r=-0.519; P=0.019) and the hemodialysis group (r=-0.350; P=0.013). This finding coincided with the study done by Zoccali *et al.* [6] who reported that high NPY was associated with systolic dysfunction and this link was stronger than and independent of that of norepinephrine. The authors also stated that NPY and norepinephrine stores are depleted in the failing myocardium and conversely the plasma concentration of NPY and NE is markedly increased in patients with heart failure and such increase is proportional to the severity of the disease indicating that these neurohormones participate in the counter-regulatory response to left ventricular failure.

The present study recorded a direct correlation between NPY level and pulse rate in the renal impairment group (r=0.575; P=0.008) and the hemodialysis group (r=0.441; P=0.001). This is consistent with the study done by Abdel Samad *et al.* [20] who showed that the endocardial endothelial cells may play an important role in the regulation of cardiac function by releasing several cardioactive factors including NPY and possess different types of NPY receptors specifically Y1 and Y2 receptors which by their activation an increase in the level of intracellular free Ca and an increase in the frequency of beating is found. The authors stated that this peptide seems to regulate excitation of these cells as well as excitation contraction coupling of ventricular cardiomyocytes.

In the present study, no correlation was found between NPY and blood pressure, although several studies showed that NPY may have a vasoconstrictor effect and subjects with high NPY level may have a blood pressure level of 2-3 mmHg higher than others [17]. In contrast, few studies reported that continuous increase in NPY level in the brain could induce hypotension and result in overall decreased metabolism and body temperatures [23]. Also Tsuda [36] proposed that over - expression of endogenous NPY may be associated with lower blood pressure due to the antiadrenergic action of NPY as it may potentiate the inhibitory effect of alpha (2) adrenergic receptor agonist on norepinephrine release, however patients included in this study were under antihypertensive treatment which may mask the effect of NPY on blood pressure.

In this study, a direct association was detected between NPY and left ventricular mass in the renal impairment group(r=0.493; P=0.027) and in the hemodialysis group(r=0.462; P= 0.001). In harmony with study results, Zoccali *et al.* [6] showed that the strong

link between NPY and left ventricular mass in renal disease is an expression of a complex relationship involving neural sympathetic influence on the heart as well as a direct effect of NPY on myocardial cells. In addition, at the cellular level, myocardial hypertrophy is based on increased mass, not number of cells as adult cardiomyocytes do not divide. Increased mass is achieved by increased synthesis of de novo protein and due to reduced degradation of existing protein. NPY attenuates protein degradation in healthy cardiomyocytes. However, NPY may increase protein synthesis which depends on the history of the pathological condition of the heart. Y2 and Y5 NPY receptors appear to be involved; therefore this may represent a novel therapeutic target for drugs designed to prevent or regress left ventricular hypertrophy [37]. In addition, it has a potent angiogenic endothelial cell activation, proliferation, migration and tube formation which may play a significant role in ischemic revascularization [23].

In light of all the above, current study findings imply that NPY is elevated in ESRD, more markedly in patients on regular hemodialysis. Such elevation may be attributed to deranged degradation as well as augmented sympathetic output which is an established trigger of CV morbidity. Thus, plasma NPY may be regarded as an emerging non-invasive factor for cardiovascular risk stratification in ESRD patients owing to its effects on heart rate, left ventricular structure and function. Current findings also indicate that ESRD patients treated by hemodialysis are at higher risk or threat of CV morbidity than ESRD patients treated conservatively as evidenced by the significantly higher plasma NPY level recorded in hemodialysis group in comparison to renal impairment group on conservative therapy.

In the current study, there was no correlation between NPY and sex; this result is in agreement with a study done by Marek *et al.* [38], who demonstrated no gender dependent differences in leptin and NPY. The present study also did not find any association between NPY and duration of dialysis as NPY is probably not removed through the dialyzer and its release does not appear to be inhibited during dialysis [39]. However, some studies showed that it could be removed by high flux dialyzer [6]. Moreover the current study did not detect correlation between NPY and body mass index. In a study done by Daghestani *et al.* [40] on the level of NPY and leptin in obese females, leptin level was positively correlated to BMI while NPY did not differ. No significant correlation was found between NPY and level of urea, creatinine, calcium, phosphorus and the lipid profile in the studied groups.

Lack of association between plasma NPY and serum CRP, mirroring inflammation, in studied ESRD patients, whether on hemodialysis therapy (r=0.210, P=0.373) or on conservative treatment (r=0.097, p=0.498) may suggest that CRP is not implicated in NPY upregulation in such condition and that they are two independent CV risk factors.

Based on the results of the current study, it can be concluded that plasma NPY is elevated in patients with renal impairment on conservative treatment and more so in patients with CRF on regular hemodialysis. Plasma NPY can be considered a nontraditional noninvasive risk factor for stratification of cardiovascular alterations complicating ESRD, specially owing to its effect on heart rate and left ventricular hypertrophy. In addition, NPY can elicit a positive effect on contraction at least initially in hypertensive patients, whereas the negative contraction effect may contribute to the pathophysiological changes leading to heart failure as evidenced by increased end systolic diameter and reduced ejection fraction. Echocardiography and plasma NPY are non invasive cost effective tools for screening and early detection of cardiovascular complications in patients with CRF, which may reduce adverse CV outcomes and improve survival in these patients.

In light of study findings, the current authors recommend the following:

- Study of the relation between NPY and atherosclerosis and clarifying its potential role in accelerating vascular events as restenosis.
- Longitudinal studies on renal patients are needed to follow the progression of cardiovascular complications and outcome in relation to serum NPY as it may be considered an easy non invasive prognostic marker for cardiovascular morbidity and mortality.
- The use of high flux dialyzer to decrease the serum level of NPY, in addition to prescription of beta blockers and the more advanced NPY receptor antagonists in CRF patients on hemodialysis.
- The use of echocardiography and plasma NPY as non invasive cost effective tool for screening and early detection of cardiovascular complications in patients with CRF.

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