Left ventricular hypertrophy and plasma Nitric oxide in hemodialysis patients

Ragai,M.F.R.Fouda[1], Yasser.M.Abdlehamid[2] and Hamdy.A.Ahmed[3]

[1]Cairo university-Department of Internal Medicine-Medical ICU- Kasr EL Aini Hospitals.
[2] Cairo university-Department of Internal Medicine-Nephrology unit- Kasr EL Aini Hospitals.
[3] Department of Biochemistry-National Research Centre-Egypt.
dyabdelhamid@kasralainy.edu.eg

Abstract: Background: Development of Left ventricular hypertrophy (LVH) in hemodialysis (HD) patients is reported in different clinical studies. The mechanisms responsible for LVH in these patients are complex and multi-factorial. Experimental studies have shown that Nitric oxide (NO) is a possible anti-hypertrophic molecule. The aim of this study was to assess prevalence of LVH and its pattern in these patients and plasma NO in these patients. **Methods:** Twenty six HD patients participated in the study. Measurement of plasma NO, and trans-thoracic echocardiographic assessment of left ventricular mass index (LVMI) and relative wall thickness (RWT) were done. LVH was diagnosed in men with LVMI>115g/m^2 and women with LVMI>95g/m^2. LVH was concentric when RWT>0.42 and eccentric when RWT<0.42. **Results:** Twenty one out of twenty six (80.8%) HD patients suffered of LVH with a mean LVMI of 191+/-78.14g/m^2. 73% of them suffered of concentric LVH, while only 7.8% of them suffered of eccentric LVH and only one patient had normal left ventricle geometry. Mean plasma NO of HD patients was significantly less than mean plasma level of healthy control subjects (6.46±1.0 microgram/dl vs 11.18±1.22 microgram/dl) and LVMI showed a significant negative correlation to plasma NO. **Conclusion:** Nearly 80% of our studied HD patients suffer of LVH , most of them suffer of concentric LVH. Mean plasma NO was significantly lower in HD patients compared to healthy control subjects. Plasma NO level was significantly negatively correlated with LVMI. Possible role of NO in the development of LVH in HD patients requires further study.
[Ragai, M.F.R. Fouda, Yasser. M. Abdlehamid and Hamdy. A. Ahmed. **Left ventricular hypertrophy and plasma Nitric oxide in hemodialysis patients.** *Life Sci J* 2012;9(4):3280-3284]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 485

Keywords: Left ventricle-hypertrophy-Nitric oxide-Hemodialysis.

1. Introduction

Excess mortality rates due to cardiovascular disease in chronic kidney disease (CKD) and end-stage renal disease (ESRD) patients had been described in epidemiological and clinical studies. It was thought that this is due to coronary artery calcification but recently attention was shifted to left ventricular hypertrophy (LVH) and fibrosis which can result in congestive heart failure (CHF) and sudden cardiac death in these patients.[1]

The development and persistence of LVH is associated with increased mortality risk.[2] ,[3] The mechanisms responsible for LVH in ESRD patients on regular hemodialyis (HD) are complex and multi-factorial. After load related factors include rise in systolic blood pressure, stiffening of the great vessels due to calcification and cross linking[4], elevation of the serum sodium concentration from hypertonic solution cause stiffening of microcirculation and reduced release of nitric oxide(NO).[5] Preload related factors including volume overload due to increase in intravascular and extracellular volume as well as hyperdynamic circulation produced by arteriovenous (A-V) fistula had been suggested. Factors not related to preload or afterload include, vitamin D deficiency, hyperphosphatemia and hyperparathyroidism, angiotensin dependent pathway, and mammalian target of rapamycin (mTOR) pathway not related to blood pressure changes have been suggested.[6]

Studies suggested that NO is a possible antihyertrophic molecule. Experimental studies have shown that nNOS or eNOS deficient mice suffer of LVH and that those animals with deficiency of both nNOS and eNOS suffer of more severe LVH. That points out to the fact that NO is a possible anti-hypertrophic molecule. [7] Thus the aim of the current study was to assess the prevalence of LVH and its pattern among HD patients using trans- thoracic echocardiography and to determine plasma NO levels in these patients.

2. Subjects and Methods:

The protocol of this study was approved by the scientific board of Nephrology division - Internal Medicine department and Committee of Research Ethics; Faculty of Medicine – Cairo University – Egypt and informed consents were obtained from all participants.

In this cross sectional study twenty six ESRD patients, undergoing regular HD via an A-V fistula three times per week in 4-hourly sessions were included in the current study. All patients were attendants of dialysis units of Kasr El-Eini hospital –

Cairo University during the period from January 2011 to October 2011. Patients with history of cardiovascular disease and those suffering of more than mild mitral or aortic valve disease were excluded.

All participants were subjected to the thorough clinical evaluation. Data regarding age, gender, body mass index, etiology of renal failure, and duration of hemodialysis were recorded. Blood sampling for assessment of blood urea, serum creatinine, calcium, phosphorus, and blood hematochrite using Hitachi 917 –Auto analyzer (Roch Diagnostics –Germany) was done.

NO metabolites (NO_2 and NO_3) in plasma of the patients were determined before the dialysis procedure with the Griess method using a Parameter™ assay commercial kit. This kit is a colorimetric assay applying nitrate reductase, which converts nitrate to nitrite, and total nitrite was measured at 540nm absorbance by reaction with Griess reagent (sulfanilamide and naphthalene ethylene diamine dihydrochloride). Amounts of nitrite in the plasma were estimated by a standard curve obtained from enzymatic conversion of $NaNO_3$ to nitrite. [8] Blood samples from healthy control subjects were obtained to assess basal NO metabolites level. Mean plasma level of serum NO measured using the same method among 30 age and gender matched healthy control subjects was 11.18±1.22ug/dl.

Two-dimensional and M-mode echocardiography were performed to all included subjects, as soon as possible following hemodialysis session. Imaging was performed with Vivid 3N (General electric) equipped with 2.5 MHz and 3.5 MHz phased pulsed array transducers. Cardiac dimensions were measured according to the guidelines of the American Society of Echocardiography using M-mode method.[9] LV mass, LV mass index, relative wall thickness (RWT) and mean wall thickness were measured using the following equations(ASE-cube formula):[10]

$$\text{LV Mass (g)} = 0.8\{1.04[([\text{LVEDD} + \text{IVSd} + \text{PWd}]^3 - \text{LVEDD}^3)]\} + 0.6$$

$$\text{LV mass index(g/m2)} = \text{LV mass/Body surface area}$$

$$\text{RWT} = \frac{2 \times \text{PWd}}{\text{LVEDD}}$$

LVEDD (mm): left ventricular end diastolic diameter, IVSD (mm): interventricular septum thickness at end of diastole, and PWD (mm): posterior wall thickness in diastole.

Women with LV mass index >95 g/m^2 and men with LV mass index >115g/m^2 suffered of LVH. LVH was considered concentric when RWT >0.42 and eccentric when RWT is <0.42.[11]

Statistical Analysis

Categorical data were summarized in the form of frequencies and percentage. Numerical data were summarized in the form of mean ± standard deviation (SD). Strength of association between variables was tested using Pearson correlation coefficient. Comparison of quantitative variables between the study groups was done using Mann Whitney U test. $P<0.05$ was considered significant. Data management and analysis were performed using Statistical Analysis Systems (Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and statistical package of social sciences (SPSS) version 13.

3. Results:

The clinical data of the studied group showed that the predominant causes of ESRD were mainly hypertension and unknown causes. One half of patients suffered of systemic hypertension or received antihypertensive medications in this study. Nearly one third of the patients are receiving beta blocker therapy (Table 1).

The laboratory data of the studied patients showed that they had high blood urea, creatinine and phosphorus levels in addition to low calcium blood levels and hematochrite percentage. Mean plasma NO among HD patients was lower than mean plasma NO among healthy control subjects reported above (Table 2). The echocardiographic data of the studied group showed that they suffer of increased interventricular septum thickness , left ventricle posterior wall thickness and LVMI (Table 3).

The echocardigraphic evaluation of the LV structure of the studied patients. revealed that 73% of them suffer of concentric LV hypertrophy, 15.4% suffer of concentric remodeling of LV, 7.8% suffer of eccentric LV hypertrophy and only 3.8% have normal LV structure (Table 4). LVMI of hypertensive patients or those receiving anti-hypertensive medication (LVMI HTN) and non hypertensive patients(LVMI nonHTN) were 206.36±69.66 g/m^2 vs 173.16±82.88g/m^2 respectively. That difference was not statistically significant (*P* value=0.21) (Figure 1).

The mean NO plasma level among HD patients was significantly lower than mean NO plasma level of healthy control subjects(6.46± 1.06 ug/dl vs 11.18±1.22ug/dl). Pearson correlation between LVMI and plasma NO showed a significant negative correlation between plasma NO and LVMI (Figure 2).

4. Discussion:

The current study results showed that 80.8% patients on regular HD for 75.23±72.17 months suffered of LVH with a mean LVMI of 191+/-78.14g/m^2. 73% of patients suffer of concentric LV hypertrophy, 15.4% suffer of concentric remodeling of LV, 7.8% suffer of eccentric LV hypertrophy and only 3.8% have normal LV structure. Mean LVMI of hypertensive patients or those receiving antihypertensive medications was not statistically

different from that of normotensive patients. Mean plasma NO of HD patients was significantly lower than that of healthy control subjects and LVMI showed a significant negative correlation between plasma NO and LVMI.

Table 1.Clinical data of included subjects of the studied group

Parameter	Value
• Age (years)	42.8±12.95
• Gender	
Males	16/26(61.5%)
Females	10/26(38.5%)
BMI (kg/m^2)	24.6±5.6
• Etiology of renal failure	
Hypertension	7/26(26.9%)
Diabetes and hypertension	3/26(11.5%)
Obstructive uropathy	3/26(11.5%)
Glomerulonephritis	2/26(7.7%)
Pyelonephritis	1/26(3.84%)
Congenital	1/26(3.84%)
Polycystic kidney	1/26(3.84%
Unknown	8/26 (30.7%)
• Dialysis duration (months)	75.23±72.17
• Co-morbidities	
Hypertension	14/26(53.85%)
Diabetes	3/26(11.5%)
Antihypertensive medications:	
• Angiotensin Converting enzyme inhibitors	1/26(3.84%)
• Beta blockers	9/26(34.6%)

Data are expressed as mean ±SD or n/n(%)

Table 2. Laboratory data of included subjects of studied group

Parameter	Value
Serum Creatinine (mg/dl)	8.12±1.49
Blood urea (mg/dl)	92.5±16.85
Serum Calcium (mg/dl)	8.65±0.82
Serum Phosphorus (mg/dl)	4.29±0.89
Calcium phosphorus product	36.5±5.44
Haematocrite(%)	34.96±3.97
Serum Nitric oxide (ug/dl)	6.46±1.1

Data are expressed as mean ±SD

Table 3.Echocardiographic data of the studied group

Parameter	Value
Left ventricle end -diastolic diameter (mm)	5.2±0.86
Left ventricle end -systolic diameter (mm)	3.26± 0.6
Inter ventricular septum thickness (mm)	1.41±0.31
Left ventricle posterior wall thickness(mm)	1.47±0.25
Relative wall thickness (mm)	0.59±0.13
Left ventricle mass(grams)	334.88±151
Left ventricle mass index (gram /m^2)	191±78.14

Data are expressed as mean ±SD

Table 4. Echocardiographic evaluation of left ventricle structure of the studied group

Parameter	Value
Normal Left ventricle	1/26(3.8 %)
Concentric left ventricle remodeling	4/26(15.4%)
Concentric Left ventricular hypertrophy	19/26(73 %)
Eccentric Left ventricular hypertrophy	2/26(7.8%)

Data are expressed as n/n(%)

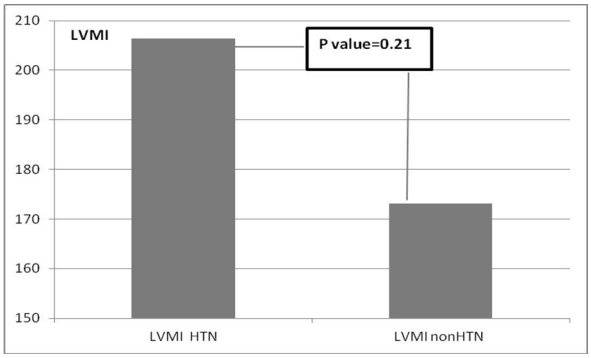

Figure 1) Comparison between left ventricle mass index in hypertensive patients(LVMI HTN) and left ventricle mass index in non hypertensive patients (LVMI nonHTN). *P* value <0.05 is considered to be significant.

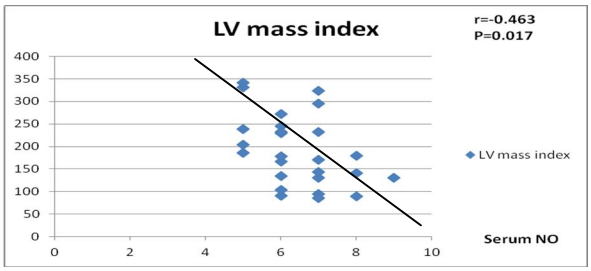

Fig 2. Person correlation between left ventricle(LV) mass index and serum Nitric oxide(NO).

Different studies reported that 68-78% of HD patients suffer of LVH. [12,13,14] The previously reported percentages are close to what we noticed in the current study. LVH pattern can be concentric or eccentric, depending upon complex intermingling of volume overload, pressure overload , and non hemodynamic factors in HD patients are responsible for the development of LVH. [15]

Zoccalli *et al.,* reported that 38% of HD patients suffer of eccentric LVH while 36% of them suffer of concentric LVH. [16] while Mannes *et al.,* studied the geometric pattern of the left ventricle among 26 HD patients, reported that eccentric LVH affected 55% of the patients and only 16% of them suffer of concentric LVH.[17]

The previously mentioned studies which suggested either equal role of volume and pressure

overload related factors or a predominant role of volume overload related factors in HD patients, while the current study showed that after load related factors appear to be more predominant, which might be due to the fact that half of patients in the current study suffer of systemic hypertension.

There was no statistically significant difference between LVMI of hypertensive HD patients and LVMI of non hypertensive HD patients in this study , which suggests that factors other than hypertension plays a role in development of LV hypertrophy. So we tried to assess plasma NO (anti-hypertrophic molecule) level in these patients. NO is released from the amino acid L-Arginine via endothelial NO synthase enzyme (eNOS),neuronal NO synthase enzyme (nNOS) and inducible NO synthases (iNOS) . All the three types of NO synthases are present in the heart.[18]

NO intracellular target is guanylate cyclase that converts guanosine triphosphate (GTP) into cyclic guanylate monophosphate (cGMP). cGMP acts via protein kinase G inhibiting a variety of pro-hypertrophic pathways including that which involve Angiotensin II , Endothelin 1, insulin, growth factors , and mitogen activated protein kinase.[19]NO in addition has a dose dependant pro-apoptotic role , high concentration of NO is needed for activation of caspases,DNA fragmentation and apoptosis.[20]

Renal failure is a state of NO deficiency. Total NO production is reduced in CKD and ESRD patients which agrees with results of the current study . NO is reduced through a variety of mechanisms in these patients, which include reduced L-Arginine, endogenous NOS inhibitors, defective renal NO production and endothelial dysfunction.[21]

As reported above, there is a significant negative correlation between LVMI of HD patients and their serum NO; these results suggest that reduced serum NO in HD patients might play a role in the development of LVH in these patients. Zocalli *et al.,* reported in a pioneer study that there was a significant correlation between serum asymmetric dimethyl arginine (ADMA) (an endogenous inhibitor of NO synthase enzyme) and LVMI in HD patients. That study was the first point to a possible role of reduced serum NO in the development of LVH in HD patients.[16]

Rasic *et al.,* reported a significant positive correlation between serum NO and LVMI in HD patients and that it is an important determinant of LV diastolic function in these patients.[22] Moreover Li and Wang reported that sustained release isosorbide mononitrate therapy in maintenance HD patients resulted in significant reduction in LVMI that appeared after 24 weeks of nitrate therapy. Their study showed that NO donors can reduce LVMI in HD patients which again supports the role of reduced serum NO in the development of LVH in these patients.[23]

Only few articles regarding the possible role of NO in the development of LVH in HD patients appeared in English literature which makes the data of the study valuable but the study got few limitations. First Cardiac magnetic resonance imaging (MRI) using gadolinium is the gold standard for assessment of LV mass, but gadolinium cannot be used in renal failure patients due to the risk of nephrogenic systemic fibrosis.[24] That was the reason for using TTE in the current study which appears to be a reasonable alternative to MRI, despite the fact that asymmetry of the LV geometry might result in errors so the results must be interpreted with caution.[25] Second although the current study showed a positive correlation between plasma NO and LVMI in HD patients , it can not prove a causal relationship due to the nature of the study.

In conclusion, LVH is a common under-recognized problem in HD patients with its consequent increase in cardiovascular mortality. Plasma NO levels correlated with echocardiographicaly determined LVMI in these patients. Study of the exact role of NO as anti-hypertrophic molecule is needed to know whether reduced NO plays actually a role in increased LVMI in these patients or it is just an innocent bystander. In the near future NO donors might play a role in prevention and treatment of that serious problem among HD patients.

Corresponding author:
Yasser.M.Abdelhamid
Internal Medicine Department.
Nephrology Unit
Faculty of Medicine-Cairo University . Cairo (Egypt).
Email : dyabdelhamid@hotmail.com

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10/11/2012