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Plasma Nitric Oxide Level in Myocardial Disorders with Left Ventricular Diastolic Dysfunction

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Nitric oxide is a free radical that is elevated in the plasma of patients with heart failure due to contractile dysfunction. This study examine the relation between plasma NO level and Left Ventricular (LV) diastolic function and its aetiology in heart failure patients in the pediatric age group. We performed echocardiographic Doppler studies in 47 patients (mean age of 6.16±2.8 years, 31 males and 16 females) with congestive heart failure. Left ventricular diastolic dysfunction was classified as either a restrictive (RFP) or non restrictive filling pattern (non-RFP). Same day venous total nitrite and nitrate levels were measured by colourimetric assay. Plasma NOx level was significantly higher in the studied patients than the control group (141±54 and 43±4 μmol/L, respectively, $p<0.001$). ROC curve found that the cut off point for plasma NOx level was 60 μmol/L to differentiate between normal children and patients with heart failure. Patients with RFP showed insignificantly higher levels of plasma NOx than the non-RFP patients. Only in muscular dystrophy patients, there were negative correlation between plasma NOx level and LV ejection fraction ($r = -0.61$, $p = 0.06$) and LV fractional shortening ($r = -0.64$, $p = 0.04$). On correlating the plasma NOx levels to the severity of heart failure by multiple linear regression analysis, the pulmonary artery systolic pressure was the only variable independently associated with an elevated plasma NOx level ($p = 0.05$). Plasma NOx level is elevated in patients with isolated diastolic heart failure. In addition, in patients with LV systolic failure, the severity of LV diastolic dysfunction determines the amount of NO production.

Key words: Nitric oxide, myocardial disorders, heart failure, diastolic dysfunction

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INTRODUCTION

Nitric Oxide (NO) is a free radical that is known to be an important determinant of vascular tone. It plays a major role in the regulation of cardiovascular homeostasis both in health and disease (Cotton *et al*., 2002; Mitsuke *et al*., 2001). Apart from controlling the coronary blood flow, there is now an emerging consensus that generally acts to fine tune and optimise cardiac pump function (Cotton *et al*., 2002). Excessive NO depresses systolic function by decreasing myocardial contractility and shortening the ejection period (Cotton *et al*., 2002). Elevated circulating levels of oxidative products of (NOx) and myocardial NO synthetase expression have been seen in patients with heart failure due to contractile dysfunction (Balat *et al*., 2003; Fukuchi *et al*., 1998). Diastolic dysfunction commonly coexists in patients with systolic heart failure (Zile *et al*., 2002). Nevertheless, some patients experience isolated diastolic heart failure i.e., heart failure in the setting of preserved systolic function (Diasp *et al*., 2001).

This study examine the relation between plasma NO level and Left Ventricular (LV) diastolic function and its aetiology in heart failure patients in the pediatric age group. Three different groups of patients with known chronic diseases of myocardium and abnormal cardiac function (thalassemia, idiopathic dilated cardiomyopathy and muscular dystrophies) were studied.

MATERIALS AND METHODS

Subjects: Forty seven patients (mean age of 6.16±2.8 years, thirty one males (66%) and sixteen females (34%)) with heart failure (New York Heart Association Functional Class II to IV) were studied. Twenty normal children (matching the patients in age and sex) were also included as a control group for the normal NO plasma level.

Patients were recruited from three outpatients clinics of Children Hospital of Cairo University Egypt. These clinics were the hematology clinic (twenty patients with thalassemia 43%), the cardiomyopathy clinic (seventeen patients with idiopathic dilated cardiomyopathy 36%) and the myopathy clinic (ten patients with muscular dystrophy 21%). The study was conducted from 1 September 2002 to 1 October 2003.

Patients were maintained on medications as angiotensin-converting enzyme inhibitors (sixteen patients (34%)), in tropics (twelve patients (26%)), diuretics (fourteen patients (30%)), aspirin (five patients 10.6%)), L-carnitive(thirty five patients (74.5%)) and dysferal (twenty patients(43%)).

Exclusion criteria: Recent history of acute heart failure in the past 4 weeks, arrhythmia, major organ dysfunction e.g., renal or hepatic, significant pulmonary disease or systemic illness, malignancy, active infection or inflammatory disease and acute myocarditis. A written consent was given by all patients or their parents.

All patients were subjected to complete clinical assessment as well as an electrocardiogram before further evaluation.

Echocardiography: Echocardiography was performed on the same day of blood sampling for plasma NO. Left ventricular volume indexes at end-systole and end-diastole were measured according to the guidelines of the American society of echocardiography. Pulse-Doppler assessment of diastolic function was performed by the interrogation of flow velocities at the mitral annulus (Yu *et al*., 2002) and confirmed by pulmonary venous inflow profile if necessary (Erbel *et al*., 2002). LV diastolic dysfunction was classified as a Restrictive Filling Pattern (RFP) or a non restrictive filling pattern (non-RFP) (Park, 1996; Zile *et al*., 2002; Vasan *et al*., 2002).

Measurements of plasma nitric oxide level by colourimetric assay: Plasma nitric oxide level was measured by the nitric oxide assay kit supplied by Assay Design Inc. Ann Arbor, M. Two milliliter of venous blood were withdrawn on sodium citrate, centrifuged at 2,000 g for 10 min and stored at -20°C till analysis. The transient and volatile nature of NO makes it unsuitable for most convenient detection methods, however, two stable breakdown products i.e., nitrate (NO_3) and nitrite (NO_2) can be easily detected by photometric methods. The technique involves the enzymatic conversion of nitrate to nitrite by the enzyme nitrate reductase followed by the colourimetric detection of nitrite as a colored azodye product of the Griesse reaction that absorbs visible light at 540 nm (Akiyama *et al*., 1997; Sheu *et al*., 2000).

Statistical analysis: SPSS (statistical package for social sciences) version 10.0 was used in data analysis. Mean and standard deviation described quantitative data. Non parametric ANOVA compared means of >2 independent group and Scheffe test made pairwise comparisons. Pearson's and Spearman Rho correlation analysis were performed to predict association of plasma nitric oxide to cardiac indices and other numerical variables. ROC (receiver operator characteristics) curve was used to choose cut off point to differentiate normal controls from cases with heart failure. Multiple linear regression analysis was performed with nitric oxide as the dependent

variables and systolic, diastolic functions, age, heart rate, sex and type of dysfunction as independent or covariates. p-value is significant at 0.05 level.

RESULTS

According to echocardiographic evaluation, all patients showed diastolic dysfunction. Seventeen of them (36.2%) had impaired systolic (ejection fraction≤50%) and diastolic functions, while 30 patients (63.8%) has isolated dysfunction (Fig. 2). The restrictive filling pattern was observed in 41 patients [26 patients with isolated diastolic dysfunction and 15 patients with systolic and diastolic dysfunction].

Figure 1 shows that plasma NOx level was significantly higher in the studied patients than the control group (141±54 and 43±4 μmol/ L, respectively, p<0.001). ROC curve found that the cut off point for plasma NOx level was 60 μmol/L to differentiate between normal children and patients with heart failure.

Figure 2 patients with RFP showed insignificantly higher levels of plasma NOx than the non-RFP patients (p = Non significant).

Table 1 shows the relation between the impaired systolic function and plasma NOx levels in the three aetiologically different heart failure patients. Only in muscular dystrophy patients, there were negative correlation between plasma NOx level and LV ejection fraction (r = -0.61, p = 0.06) and LV fractional shortening (r = -0.64 , p = 0.04).

Table 2 on correlating the plasma NOx levels to the severity of heart failure by multiple linear regression analysis, the pulmonary artery systolic pressure was the only variable independently associated with an elevated plasma NOx level (p = 0.05).

There was no significant correlation of plasma level of NOx and either the aetiology of heart failure shown in (Fig. 1) or the medications received by the patients.

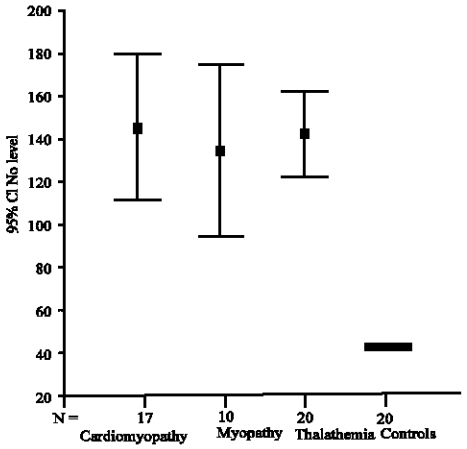

Fig. 1: Nitric oxide level among all study groups No. = Nitric Oxide

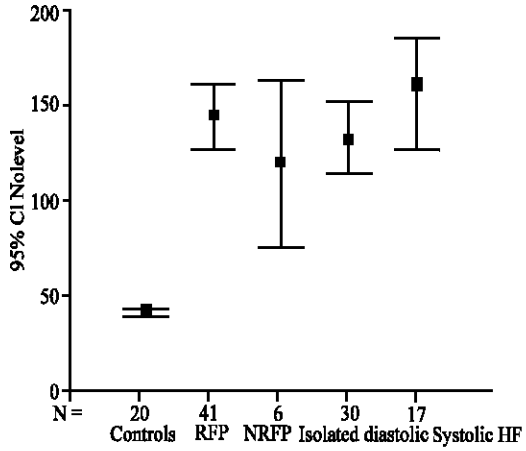

Fig. 2: Nitric oxide level according to types of heart failure, RFP = Restrictive Filling Pattern, NRFP = non Restrictive Filling Pattern, HF = Heart Failure, NO =Nitric Oxide

Table 1: Correlation between plasma nitric oxide level and individual systolic and diastolic parameters

	Cardiomyopathy n =17		Muscular dystrophy n = 10		Thalassemia n = 20		All groups total: 47	
Parameters	r	p-value	r	p-value	r	p-value	r	p-value
E velocity	0.42	NS	-0.13	NS	-0.27	NS	-0.03	NS
A velocity	-0.21	NS	-0.09	NS	-0.52	0.02	-0.29	NS
E/A ratio	0.43	NS	0.03	NS	0.07	NS	0.20	NS
Deceleration time of E	0.30	NS	-0.39	NS	0.08	NS	-0.004	NS
LVEDD	0.02	NS	-0.27	NS	-0.30	NS	-0.05	NS
LVESD	0.14	NS	-0.28	NS	-0.27	NS	0.05	NS
LVEF	0.04	NS	-0.61	NS	0.17	NS	-0.08	NS
LVFS	-0.28	NS	0.61	0.06*	0.18	NS	-0.19	NS
			-0.64	0.04*				

A velocity = Transmittal peak atrial filling velocity; E velocity = Transmittal peak early filling velocity; E/A ratio = Ratio of transmittal peak early to atrial filling velocity; LVEDD = Left Ventricular End Diastolic Dimension; LVESD= Left Ventricular End Systolic Dimension; LVEF = Left Ventricular Ejection Fraction; LVFS= Left Ventricular Fractional Shortening

Table 2: Multiple linear regression analysis comparing the correlation between plasma nitric oxide level and individual variables

Variable	β	p-value
E velocity	-0.78	0.14
A velocity	0.29	0.54
E /A ratio	0.88	0.19
Deceleration time of E	0.01	0.96
LVEDD	-0.46	0.19
LVESD	1.5	0.13
PASP	-0.29	0.05*
Age	-0.02	0.09
Sex	0.13	0.39
Heart rate	0.08	0.66

P = 0.05*, Abbreviations as in Table 1

DISCUSSION

There is now good evidence that NO has important autocrine/paracrine effects in the myocardium, in general serving to optimize and fine tune the cardiac function through actions on inotrope stat, excitation-contraction coupling, diastolic function, heart rate and β adrenergic responsiveness It is clear that the biological activity of NO is altered during human heart failure (Cheuk-Man *et al*., 2001).

In this study the plasma NOx level was significantly higher in the studied patients than the control group ($p<0.001$). The elevation of circulating NOx could be a consequence of increased cardiac production, as NO is carried away by hemoglobin as well as by the amino acid glutathione and cysteine. It has been demonstrated that there is beat-to-beat cardiac NO production in response to mechanical stimuli which is maximal at the mid diastole in isolated heart preparation (Pinsky *et al*., 1997). In the heart, microvascular and endocardial cells were the main sources of load-dependent cardiac NO, through the activation of endothelial NO synthase (Pinsky *et al*., 1997; Stefan *et al*., 2004). There has been evidence that the stable end product of NO (i.e., nitrate) was significantly increased in patients with chronic heart failure (Balat *et al*., 2003). In an in vitro study, inducible NO synthase expression was found to be increased in ventricular myocytes isolated from the severely failing heart (Fukuchi *et al*., 1998).

In this study, there was a significant elevation of plasma NO level in patients with isolated LV diastolic dysfunction, as well as those with combined systolic and diastolic dysfunction ($p<0.001$). In conjunction with the results of present study it has been speculated that elevation of plasma NOx in patients with heart failure, especially in those with isolated diastolic heart failure, is a compensatory response to the elevated LV filling pressure. This is supported by the fact that the basal cardiac secretion of NO is important in the maintenance of diastolic function (Mitsuke *et al*., 2001) as well as infusion of NO to patients with LV hypertrophy, which has beneficial hemodynamic effects on the parameters of diastolic function (Mitsuke *et al*., 2001; Matter *et al*., 1999).

In contrast, depending on the amount and mechanism of NO production, excess NO production can be detrimental to the heart. Studies have found that cytokine inducible NO synthase was expressed in cardiac myocytes with contractile failure of various etiologies and overproduction of NO is likely a result (Mitsuke *et al*., 2001; Thibaud *et al*., 2004).

Excessive NO has been shown to depress contractile function, can be cytotoxic and can induce apoptosis. Immunological response to heart failure result in endothelial and myocyte dysfunction through oxidative stress mediated apoptosis (Ferrari *et al*., 2004). These events, however, are unlikely to occur in isolated diastolic heart failure in which contractile function is preserved and myocyte damage is minimal. Other than the ventricle, atrial production of NO can not be excluded as the plasma NOx level has also been found to correlate with left atrial size (Mitsuke *et al*., 2001).

In this study Patients with RFP had higher plasma NOx levels than those with non-RFP. On ROC curve the cut off point of plasma NOx level was at 152 μmol/L to differentiate between RFP and a non-RFP patients. All patients above this level had a RFP. RFP is more prevalent in systolic heart failure with left ventricular diastolic dysfunction. It signifies more advanced heart failure with higher filling pressure and decreased compliance in both left atrial and left ventricle, as well as a worse prognosis. (Diasp *et al*., 2001; Erbel *et al*., 2002).

In the present study there were negative correlation between plasma Nox level and LV ejection fraction ($r = -0.61$, $p = 0.06$) and LV fractional shortening ($r = -0.64$, $p = 0.04$) in muscular dystrophy patients.

In a study done by Node *et al.* (2000) patients with mild to severe heart failure underwent right and left heart catherization, the generation of NOx confirmed by the increase in the level in the coronary sinus and therefore, the difference between coronary sinus and ascending aorta. These studies confirmed the cardiac source of production of NO in systolic heart failure, its correlation with coexisting diastolic dysfunction and overproduction of NO in isolated diastolic heart failure has not been demonstrated.

NO may also be synthesized from non cardiac sources, such as in skeletal muscle of patients with severe systolic heart failure (Riede *et al*., 1998). Peripheral vascular endothelial NO production does not account for

these changes, as endothelial dysfunction secondary to reduced endothelial NO synthesis had been previously described by Katz (1997).

In this study on correlating the plasma NOx levels to the severity of heart failure by multiple linear regression analysis, the pulmonary artery systolic pressure was the only variable independently associated with an elevated plasma NOx level ($p = 0.05$). It signifies that heart failure is advanced in these patients and the right ventricular pressure may exceeds the systemic pressure with bad prognosis (Park, 1996).

In view of the role of the speculated role of NO in heart failure, NO targeted therapy is a potentially useful therapeutic modality in these patients, which is exemplified by the use of NO in LV hypertrophy (Matter *et al*., 1999). Inhaled nitric oxide has shown promise for acute right ventricular failure (Wasson *et al*., 2004). L-NG-mono methyle-arginine (L-NMMA), an NOS inhibitor blocks negative inotropic effects of NO and aminoguanidine (a selective inducible NO synthase inhibitor) is used in early cardiac allograft rejection (Worrall *et al*., 1997). The different mechanisms by which NO results in these contrasting effects seen in CHF may involve decreases and increases in oxidative stress, respectively.

CONCLUSIONS

It be we concluded that, plasma NOx level is elevated in patients with isolated diastolic heart failure. In addition, in patients with LV systolic failure, the severity of LV diastolic dysfunction determines the amount of NO production. NO targeted therapy is a potentially useful modality in heart failure and the use of aminogunidine (a selective inducible NO synthase inhibitor) in early cardiac allograft rejection.

Further studies are warranted to explore the relation between NO and other markers for heart failure and to define which children may benefit most from NO therapy and to determine the effects of this therapy on prognosis and long term survival.

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