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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 examines 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 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 levels were significantly higher in the patient group than in the control group (141 ± 54 µmol/l and 43 ± 4 µmol/l, respectively; $p < 0.001$). ROC curves found that the cut-off point for plasma NOx levels 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 ($p = n. s.$).

Only in muscular dystrophy patients, the correlation between plasma NOx levels and LV ejection fractions ($r = –0.61$; $p = 0.06$) and LV fractional shortenings ($r = –0.64$; $p = 0.04$) was negative.

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 levels are 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. ***J Clin Basic Cardiol 2006; 9 (online): 23–6.***

Key words: *nitric oxide, myocardial disorders, heart failure, diastolic dysfunction*

Nitric oxide (NO) is a free radical known to be an important determinant of vascular tone. It plays a major role in the regulation of cardiovascular homeostasis both in important health and disease [1, 2].

Apart from controlling the coronary blood flow, there is now an emerging consensus that generally acts to fine-tune and optimise cardiac pump function [1]. Excessive NO depresses systolic function by decreasing myocardial contractility and shortening the ejection period [1]. 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 [3, 4]. Diastolic dysfunction commonly co-exists in patients with systolic heart failure [5]. Nevertheless, some patients experience isolated diastolic heart failure, i.e. heart failure in the setting of preserved systolic function [6].

This study examines 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 dystrophy) were studied.

Methods

Subjects

47 patients (mean age 6.16 ± 2.8 years; 31 males [66 %], 16 females [34 %]) with heart failure (NYHA II–IV) were studied. 20 healthy children (matching the patients in age and sex) were also included as a control group for the normal NO plasma levels.

Patients were recruited from three outpatient clinics of the Childrens' Hospital of Cairo University. These clinics were the hematology clinic (20 patients with thalassemia [43 %]), the cardiomyopathy clinic (17 patients with idiopathic dilated cardiomyopathy [36 %]), and the myopathy clinic (10 patients with muscular dystrophy [21 %]).

Patients were maintained on medications such as angiotensin-converting enzyme inhibitors (16 patients [34 %]), intropics (12 patients [26 %]), diuretics (14 patients [30 %]), aspirin (5 patients [10.6 %]), L-carnitine (35 patients [74.5 %]) and dysferal (20 [43 %]).

Exclusion Criteria

Patients were excluded if they had a 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. 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 by a 2-dimensionally guided M-mode method according to the guidelines of the American Society of Echocardiography [7]. The ejection fraction was calculated using the modified Simpson's rule. Pulse-Doppler assessment of diastolic function was performed by interrogation of flow velocities at the mitral annulus [8], and confirmed by pulmonary venous inflow profile, if necessary [9]. The average of ≥ 3 consecutive beats was taken. LV diastolic dysfunction was classified as a restrictive filling pattern (RFP) (defined as early to atrial filling [E/A] ≥ 2 or E/A = 1–2 and deceleration time of early filling [DT] < 110 ms), or a non-restrictive filling pattern (non-RFP;

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defined as E/A ratio < 1 or E/A = 1–2 and DT > 275 ms, normal transmitral pattern but abnormal pulmonary venous flow profile [reverse in systolic to diastolic forward ratio]) [5, 10, 11].

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. 2 ml of venous blood were withdrawn on sodium citrate, centrifuged at 2,000 g for 10 minutes, and stored at –20 °C until 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 enzyme nitrate reductase followed by colourimetric detection of nitrite as a colored azodye product of the Griesse reaction [12, 13].

Statistical Analysis

SPSS (Statistical Package for Social Sciences) version 10.0 was used in data analysis. Mean and standard deviations described quantitative data. Non-parametric ANOVA compared means of > 2 independent groups and Scheffe test made pairwise comparisons. Pearson's and Spearman Rho correlation analyses were performed to predict association of plasma nitric oxide to cardiac indices and other numerical variables. The ROC (receiver operator characteristics) curve was used to choose a 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. 17 of them (36.2 %) had impaired systolic (ejection fraction ≤ 50 %) and diastolic functions, while 30 patients (63.8 %) had isolated dysfunction (Fig. 1). The restrictive filling pattern was observed in 41 patients (26 patients with isolated diastolic dysfunction and 15 patients with systolic and diastolic dysfunction).

Figure 2 shows that plasma NOx levels were significantly higher in the patient group than the control group (141 ± 54 µmol/l and 43 ± 4 µmol/l, respectively; p < 0.001). ROC curve found that the cut-off point for plasma NOx levels was 60 µmol/l to differentiate between healthy children and patients with heart failure.

According to Figure 1, patients with RFP showed insignificantly higher levels of plasma NOx than non-RFP patients (p = n. s.).

Table 1 shows the relation between impaired systolic function and plasma NOx levels in the three aetiologically

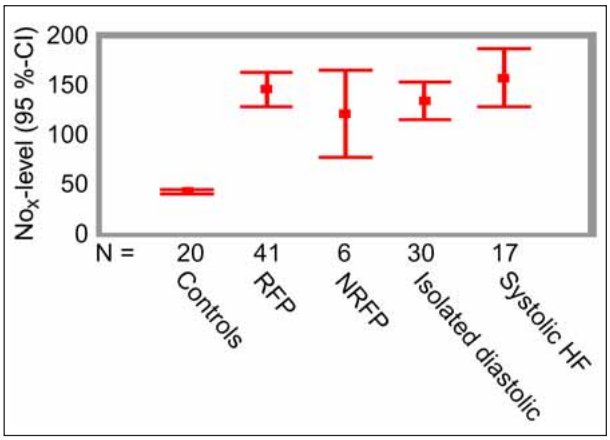

Figure 1. Nitric oxide levels according to types of heart failure

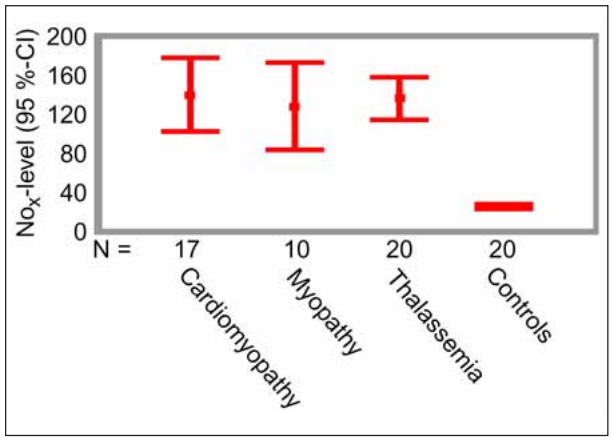

Figure 2. Nitric oxide levels among all study groups

Table 1. Correlation between plasma nitric oxide levels and individual systolic and diastolic parameters

Parameters	Cardiomyopathy (n = 17)		Muscular dystrophy (n = 10)		Thalassemia (n = 20)		All groups (total: 47)	
	r	p-value	r	p-value	r	p-value	r	p-value
E-velocity	0.42	n. s.	–0.13	n. s.	–0.27	n. s.	–0.03	n. s.
A-velocity	–0.21	n. s.	–0.09	n. s.	–0.52	0.2	–0.29	n. s.
E/A-ratio	0.43	n. s.	0.03	n. s.	0.07	n. s.	–0.20	n. s.
Deceleration time of E	0.30	n. s.	–0.39	n. s.	0.08	n. s.	–0.004	n. s.
LVEDD	0.02	n. s.	–0.27	n. s.	–0.30	n. s.	–0.05	n. s.
LVESD	0.14	n. s.	–0.28	n. s.	–0.27	n. s.	0.05	n. s.
LVEF	0.04	n. s.	–0.61	0.06*	0.17	n. s.	–0.08	n. s.
LVFS	–0.28	n. s.	–0.064	0.04*	0.18	n. s.	–0.19	n. s.

E-velocity = transmitral peak early-filling velocity; A-velocity = transmitral peak atrial filling velocity; E/A-ratio = ratio of transmitral 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; *p < 0.05 was considered significant

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

Varibale	beta	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	1.50	0.13
PASP	–0.29	0.05*
Age	–0.02	0.09
Sex	0.13	0.39
Heart rate	0.08	0.66

* $p < 0.05$ was considered significant; PASP = pulmonary artery systolic pressure; for other abbreviations see Table 1

different heart failure patients. Only in muscular dystrophy patients, there were negative correlations 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 (Fig. 2) or the medications received by the patients.

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 beta-adrenergic responsiveness. It is clear that the biological activity of NO is altered during human heart failure [14].

In our study, there was a significant elevation of plasma NO levels in patients with isolated LV diastolic dysfunction, as well as those with combined systolic and diastolic dysfunction. It also showed that the coexisting severity of LV diastolic dysfunction, rather than LV systolic dysfunction itself, correlates with plasma NOx level. Patients with RFP had higher plasma NOx levels than those with non-RFP. On the ROC curve, the cut-off point of plasma NOx levels 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 [6, 9].

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 [15].

In the heart, microvascular and endocardial cells were the main sources of load-dependent cardiac NO, through the activation of endothelial NO synthase [15, 16]. There is evidence that the stable end product of NO (i.e. nitrate) is significantly increased in patients with chronic heart failure [3]. In an *in vitro* study, inducible NO synthase expression was found to be increased in ventricular myocytes isolated from the severely failing heart [4].

In one study, patients with mild to severe heart failure underwent right and left heart catherization [17]. 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 [17]. 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 have not been demonstrated.

In conjunction with the results of the 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 compansatory 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 [2], as well as infusion of NO to patients with LV hypertrophy, which has beneficial hemodynamic effects on the parameters of diastolic function [2, 18].

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 [2, 19].

Excessive NO has been shown to depress contractile function, can be cytotoxic and can induce apoptosis. Immunological response to heart failure results in endothelial and myocyte dysfunction through oxidative stress-mediated apoptosis [20]. 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 [2]. Lastly, NO may also be synthesized from non-cardiac sources, such as in skeletal muscles of patients with severe systolic heart failure [21]. Peripheral vascular endothelial NO production does not account for these changes, as endothelial dysfunction secondary to reduced endothelial NO synthesis had been previously described [22].

Regarding 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 [17]. Inhaled nitric oxide has shown promise for acute right ventricular failure [23]. L-NG-mono methyl-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 [24]. The different mechanisms by which NO results in these contrasting effects seen in CHF may involve decreases and increases in oxidative stress, respectively.

Acknowledgements

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