

hypertension. Thus, the aim of this study is to clarify the relation between pulmonary hypertension and NO levels in patients with congenital heart defects.

METHODS

The study was performed on 40 consecutive patients with congenital heart disease who underwent cardiac catheterization with informed consent. Routine cardiac catheterization was carried out by percutaneous technique via the femoral vein and artery. The cardiac output, pulmonary and systemic flow (Q_p and Q_s), Q_p/Q_s ratio, shunt, and systemic and pulmonary resistance (PVR) were calculated according to Fick's principle. Patients were divided into two groups according to their pulmonary arterial pressure. In group I (Pulmonary hypertensive group, n:20), the mean pulmonary arterial pressure was higher than 25 mmHg and in group II (pulmonary normotensive group, controls, n:20), lower than 25 mmHg. Blood samples were taken from the right atrium (RA), right ventricle (RV), pulmonary artery (PA), left ventricle (LV) and aorta (Ao). Two cc blood samples were taken from each site and placed in deionised tubes. The NO levels were measured by spectrophotometric method.

Nitric oxide assay: Plasma samples were deproteinised before the assay. Briefly, for every 200 μ l sample, 400 μ l of 0.5 N sodium hydroxide and 400 μ l of 10% zinc sulfate were added.; The samples were vortexed and centrifuged at 25000 s at 4 C° for 5

minute. NO metabolites (nitrate and nitrite) were assayed by first reducing nitrate to nitrite as the first step. Nitrate reductase (0,05 u/ml) along with NADPH (90 μ mol/L) and FAD (3, 12 μ mol/L) were added to each sample to convert nitrates and nitrites. Nitrite production was then determined with the Greiss reactions. For this study an aliquot at 400 μ l at plasma or water blank was mixed with 200 μ l at 0, 32 mol/L potassium phosphate buffer PH: 7, 5 and 40 μ l at nitrate reductase with co-factors. To the sample mixture 160 μ l of Greiss reagent (% 10 sulfonilamide and % 1 naphthylethylenediamine dihydrochloride in % 85 phosphoric acid) was added. The mixture was incubated for 10 minute at room temperature and the absorbance was read at 500 mm. Concentrations were determined by comparing them with a standart solution of sodium nitrate (1–100 μ mol/L) (5). The interassay and intrassay coefficients of variation for the Greiss reaction were <8.0% and <4.0%. In addition, to determine percent conversion of nitrate to nitrites, each assay contained a standart curve of 0.25 to 64 μ mol/L of nitrate. The overall percent conversion was $102.1\% \pm 0.69\%$.

Statistical analysis

The result are expressed as median values with their ranges. Comparison between control subjects and patients with pulmonary hypertension were done by Mann-Whitney U test. Multiple linear regression analysis was applied to

correlation between the hemodynamic values and NO levels. P values of less than 0.05 was considered as statistically significant.

RESULTS

The hemodynamic measurements were shown in Table I, and NO levels in Table II. The right ventricle pressure (70 mmHg) and mean pulmonary arterial pressure (49 mmHg) of the patients in group I were significantly higher than those of group II (30 and 14.5 mmHg respectively) ($P < 0.05$). PVR (2 U/m²) and Qp/Qs (2/1)

in group I were significantly higher than in group II (1 U/m² and 1/1 respectively) ($P < 0.05$). There was statistically significant difference in the pulmonary NO levels between group and group II ($P < 0.05$). Pulmonary NO level in group I was lower than group II. There was also a positive correlation between pulmonary vascular resistance and pulmonary arterial pressure ($P < 0.05$). Pulmonary NO levels were negative correlated with pulmonary arterial pressure ($P < 0.05$). Aorta NO levels were positively correlated with left ventricle pressures.

TABLE I: The hemodynamic parameters in cardiac chambers in patients with pulmonary hypertension (group I) and without pulmonary hypertension (group II).

	Group I	Group II	P value
Age (year)	5, 5 (11-1)	5, 5 (12-1)	>0.05
RAp (mmHg)	4, 5 (10-3)	3, 5 (6-2)	>0.05
RVp (mmHg)	70 (110-38)	30 (60-20)	<0.05*
PAp (mmHg)	49 (92-25)	14, 4 (23-8)	<0.05*
LVp (mmHg)	95 (145-50, 0)	102, 5 (165-70)	>0.05
Ao p (mmHg)	66 (105-45)	85 (100-52)	>0.05
PVR (U/m ²)	2, 0 (17-0, 0)	1 (1-0, 0)	<0.05*
Qp/Qs	2, 0 (5-1, 0)	1 (2-1, 0)	<0.05*

*Statistically significant

TABLE II: The NO levels in cardiac chambers in patients with pulmonary hypertension (group I) and without pulmonary hypertension (group II).

	Group I	Group II	P value
RA no $\mu\text{mol/L}$	27, 5 (41-13)	30 (46-19)	>0.05
RV no $\mu\text{mol/L}$	28 (51-15)	31 (42-23)	>0.05
PA no $\mu\text{mol/L}$	21, 0 (26, 0-11, 0)	30 (41-11)	<0.05*
LV no $\mu\text{mol/L}$	29, 5 (44-20)	28, 5 (39-16)	>0.05
Ao no $\mu\text{mol/L}$	31, 5 (41, 0-21, 0)	29, 5 (61-15)	>0.05

DISCUSSION

NO is synthesized by many kinds of cells in mammalian tissues, including vascular endothelial cells from the amino L-arginine by the enzymatic action of NO synthase. In the cardiovascular system, the release of NO has an important role in regulating vasomotor tone and maintaining the fluidity of the blood (1, 2). Such studies implicate NO as an important mediator of pulmonary blood flow. Increased vascular resistance or vasospasm need not be due to an increase in vasoconstrictor effects, but may reflect a loss of NO mediated basal vasodilator tone. This may underlie the intricacy of alternations in local and regional microvascular perfusion in pathological states, such as pulmonary hypertension (1, 2). In our study, we have investigated the NO levels in patients with congenital heart defects and pulmonary hypertension. We did not find any study about the congenital heart disease with pulmonary hypertension in the literature, except for those done by using inhaled NO in patients with congenital heart defect. Inhaled NO was shown to be a selective pulmonary vasodilator in lambs. NO was reported to decrease pulmonary vascular resistance in adults with explained pulmonary hypertension or cardiac disease (6). How NO relaxes smooth muscles still remains to be clarified. Roberts et al (7) was the first to study in patients with congenital heart lesions during cardiac catheterization. Wessel et al (8) used inhaled NO as a probe of postoperative

endothelial function. Winberg et al (9) studied 20 patients with ASD and VSD and preoperative endothelial function. Andrew et al (10) have studied the effect of inhaled NO in children with congenital mitral stenosis and elevated pulmonary vascular resistance. Allman et al (4) have investigated the effect of NO on pulmonary arterial pressures of children with congenital heart defect. All authors have shown that inhaled NO was useful in patients with congenital heart defects and pulmonary hypertension.

In this study, we measured the NO concentrations in blood samples. It is difficult to measure NO in blood samples because NO is not stable and very quickly degrades into nitrate or nitrite in blood by hemoglobin. For this reason, we have found few studies on NO levels in the literature; Adachi et al (3) measured the NO production during exercise in chronic heart disease, and Areas-Diaz et al (11) investigated the NO levels in lung cancer patients. We did not come across any study related with NO levels in patients with congenital heart disease and pulmonary hypertension. In our study, the NO levels in patients with congenital heart defect and pulmonary hypertension were statistically different from those in patients with congenital heart defects without pulmonary hypertension. Pulmonary NO levels were low in children with pulmonary hypertension. It is now clear that increased vascular resistance or vasospasm need not be due to an increase in vasoconstrictor effects, but may reflect a loss of NO-mediated basal vasodilator tone and a decrease of NO level (1).

In conclusion, we have found that pulmonary NO levels in patients with pulmonary hypertension and congenital heart defects were low. For this reason, inhaled NO is still used in patients with congenital heart defects and pulmonary hypertension but, new studies are needed on the NO levels in congenital heart defect with pulmonary hypertension.

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