CORRELATION OF PLASMA ENDOTHELIN-1 LEVELS WITH PULMONARY HYPERTENSION AFTER INHALED NITRIC OXIDE THERAPY

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Background: Variable response to inhaled nitric oxide (iNO) therapy in patients with mitral stenosis (MS) having pulmonary hypertension (PH) has been documented in early studies. The objectives of this study were to measure plasma Endothelin-1 (ET-1) levels in those patients and to correlate them with pulmonary vascular indices after iNO therapy. It was Quesi-experimental study. **Methods:** Thirty patients with mitral or mixed mitral and aortic valve disease with severe pulmonary hypertension and enrolled for valve replacement surgery were included. Before the replacement, baseline pulmonary vascular indices and cardiac output were recorded. After the surgery, 10–20 ppm iNO was administered for 1 hour and all the parameters were again recorded. Patients were grouped into responders and non responders on the basis of % reduction in Pulmonary Vascular Resistance (PVR) after iNO therapy. Plasma ET-1 levels were measured in both groups by ELISA before and after the iNO therapy. Paired sample *t*-test was used to compare mean values for significance. The correlations between variables were then calculated by using Pearson's coefficient. **Results:** The plasma ET-1 levels were very high in all patients. They reduced in responders after iNO therapy; non-responders paradoxically showed significant increase in the levels of ET-1 after iNO therapy. Moreover, a positive correlation was observed in plasma ET-1 levels and post operative levels of PVR. **Conclusion:** The correlation of changes in PVR and plasma ET-1 levels in responders suggests that high plasma ET-1 is a key mediator of poor response in PH secondary to MS, after iNO therapy.

Keywords: Mitral Stenosis, Pulmonary Hypertension, Inhaled Nitric Oxide

INTRODUCTION

The physiological role of endogenous NO has been one of the fastest growing areas of medical research during the last few years. It plays an important role in the regulation of vascular tone and mesenchymal cell growth.[1] It is a selective pulmonary vasodilator that effectively reduces PAP by causing vascular smooth muscle relaxation after being released by the vascular endothelium.[2] It was welcomed in the ITC settings for the treatment of post cardiac surgery pulmonary hypertension as it was very conveniently administered through inhalation via ventilator setting.[3] We have documented a favorable response to inhaled nitric oxide therapy after corrective surgery in patients with severe MS and PH. however; it was observed that some patients show persistent pulmonary hypertension after valvular heart surgery indicating a poor response to inhaled nitric oxide (iNO).[4]

Studies have demonstrated the interaction between NO and a very potent vasoconstrictor ET-1 in the vascular endothelium. Adel Giaid and his colleagues showed increased expression of ET-1 in the pulmonary arteries of patients with primary and secondary pulmonary hypertension. They also demonstrated diminished expression of endothelial nitric oxide synthase, the enzyme responsible for generating NO, in patients with the same disease.[5] In another study by the same author on lung tissue specimens of patients with primary or secondary pulmonary hypertension the expression of NOS-I was similar in normal and diseased lungs, while abundant expression of ECE-1 was present in diseased pulmonary vessels, which may contribute to the pathogenesis of vascular remodelling in pulmonary hypertension.[6]

But the data on the levels of ET-1 in patients with pulmonary hypertension (PH) secondary to MS is rare. Moreover, no such study is thus far available that shows the effects of inhaled nitric oxide on the endothelin-1 levels in PH secondary to MS.

This study was carried out to measure plasma ET-1 levels in patients with severe MS having Secondary Pulmonary Hypertension and undergoing mitral valve replacement. We will also correlate the plasma ET-1 levels with pulmonary vascular indices after inhaled Nitric Oxide (iNO) therapy.

MATERIAL AND METHODS

This study was carried out at intensive care unit (ICU) of Armed Forces Institute of Cardiology/ National Institute of Heart Diseases (AFIC/NIHD) Rawalpindi. Biochemical estimation of plasma Endothelin-1 was performed by enzyme linked immunosorbent assay (ELISA) at Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

No patient had a history of malignancy, renal or liver disease, diabetes mellitus, hypertension, hyperlipidemias, infectious disease, deep venous thrombosis, pulmonary embolism, or recent surgery.

Thirty patients with symptomatic, moderate to severe Mitral stenosis (mitral valve area 1.05±0.14 Cm^2) were enrolled in the study. Inclusion criteria included Systolic Pulmonary Artery Pressure ≥80 mmHg on echocardiography done preoperatively. Out of 30, 16 patients were having sinus rhythm while 14 patients had atrial fibrillation. Written and informed consent was taken from all the patients after fully explaining the procedures. The protocol was reviewed and approved by the Ethics Review Committee of AFIC Rawalpindi.

Baseline hemodynamic parameters i.e. Central Venous Pressure (CVP), Pulmonary Capillary Wedge Pressure (PCWP) and Pulmonary Artery Pressure (PAP) were recorded with Swan Ganz Catheter (7.5 French Arrow) one day before the corrective surgery.[7] A mild sedative was administered through intravenous (I/V) route before the catheterization, to keep the patient relax. The catheter was introduced through a central venous line (Arrow.7.5 French, *Reading,Pennsylvania*) inserted through the right/left neck vein observing strict antisepsis. . During its passage various pressures were recorded through a pressure transducer connected to the catheter. The catheter was left in place for subsequent recording of hemodynamic parameters after Nitric Oxide administration. Pulmonary vascular resistance ($dyne.sec.cm^{-5}m^2$) was calculated with the help of standard formula:

$$\frac{\text{Mean pulmonary artery pressure} - \text{pulmonary capillary wedge pressure}}{\text{Cardiac output}} \times 80$$

Through an arterial cannula (18 G) introduced through the right radial artery, the Lithium Dilution Cardiac Output (LiDCO) monitor was attached to record Heart rate (HR), Stroke Volume(SV), & Systemic Arterial Pressure(SAP) and Cardiac Output (CO).[8] It senses the hemodynamic changes on beat to beat basis and is a highly sensitive machine used in ICU settings. For each hemodynamic variable three readings were taken and finally means were calculated.

The next day, at the arrival of patient from the operation theatre after mitral or double valve replacement, inhaled nitric oxide was administered through the inspiratory limb of a Servo 300-A ventilator (Siemens-Elema AB, Electromedical Systems Division) in a dose of 10–20 ppm to all patients. INO was started at 5 ppm and then gradually increased to 10 and 20 ppm.[9] The ventilator was set on SIMV (synchronized intermittent minute ventilation) pressure support. Tidal volume was set on 10 ml/Kg and FiO_2 was kept at 50%. Plateau airway pressure was kept at 25–30 mmHg and Positive End Expiratory Pressure (PEEP) at 5 mmHg. After 1 hour of iNO administration, all the study parameters were again recorded.

For the estimation of ET-1, 3 ml of venous blood was drawn into pre-chilled plastic tubes containing K^3 EDTA (K^3 ethylene diamine tetra acetic) acid), from antecubital vein under minimum pressure using 5 cc syringe, with patient in the supine position. Tubes containing mixture of blood and EDTA were immediately put on the ice and centrifuged at -4 ºC in cold centrifuge at 3000 revolutions per minute, for 20minutes and the extracted plasma was shifted into plain tubes and stored at -70 ºC for measurement of ET-1 in the plasma.

Plasma endothelin-1 levels were estimated by sandwich ELISA, using commercially available Endothelin ELISA kit manufactured by Biomedica Medicine products GmbH & Co KG, U.K. (CAT. No BI-20052).[10] The minimum detection limit of ET-1 using this kit is 0.62 ηg/ml with no cross-reactivity with endothelin.

Plasma samples, standards & controls were added to a 96 well microplate and mixed with monoclonal mouse anti ET-1 antibody by swirling gently. The samples were then covered tightly and incubated at room temperature (18–26 ºC) overnight. After that wells were washed with wash buffer.

In the next step, enzyme (horseradish peroxidase) labelled second antibody was added into each sample and was covered tightly and incubated for 1 hour at 37 ºC in a shaker. After that wells were again washed and substrate (Tetramethylbenzidine solution) was added into each sample and incubated for 30 minutes at room temperature in the dark. Finally stop solution was added into each well and shook well in a shaker. Absorbance was measured immediately at 450 ηm with reference 620 ηm.

A standard curve was obtained with the use of mean absorbance values of the included ET-1 standards, and the ET-1 concentration in all the unknown samples were then calculated with linear regression. All the standards and samples were tested in duplicate.

The data was entered into statistical package SPSS version 10. Descriptive statistics were used to calculate Means and Standard Deviation of all variables. Paired sample *t*-test was used to compare hemodynamic variables, i.e., pulmonary artery pressure and pulmonary vascular resistance and ET-1 levels in responders and non-responders of iNO, before and after its administration. Correlation analysis was done to look for the degree of correlation between plasma ET-1 levels and pulmonary vascular resistance and pulmonary artery pressure. The values were interpreted as follows:

r= 0: No correlation
r= +1: Perfect positive correlation
r= -1: Perfect negative relationship

RESULTS

Twenty-one women and 9 men underwent corrective surgery for mitral stenosis with secondary pulmonary hypertension. Out of these, 21 (70%) had isolated mitral stenosis and 9 (30.0%) had mitral stenosis

combined with aortic regurgitation. Two of these patients had tricuspid regurgitation and underwent tricuspid repair along with mitral valve replacement.

After the valve replacement the patients were started on inhaled nitric oxide for one hour. Response to iNO provided two categories of subjects: Responders showed ≥40.0% reduction in pulmonary vascular resistance whereas non-responders showed ≤40.0% reduction. (Figure-1)

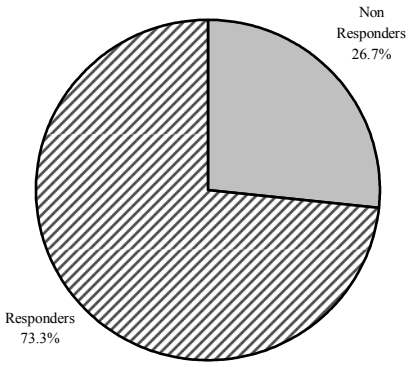

Figure-1: Distribution of subjects on the basis of response to inhaled Nitric Oxide into Responders (n=22) and Non Responders (n=8).

Table-1 shows the comparison of plasma endothelin-1 levels between responders and non responders before and after iNO administration. Plasma endothelin-1 levels fell from 0.627±0.20 to 0.466±8.81 fmol/l after iNO administration in responders, whereas in non responders the post iNO levels rose from 0.56±7.99 to 0.68±0.12 fmol/l (p<0.001).

The post iNO values of mean pulmonary artery pressures and pulmonary vascular resistance showed significant differences between the two groups (p<0.001). The mean pulmonary artery pressure dropped from 65.73±14.87 mmHg to 28.09 ± 6.93 mmHg in responders whereas the non-responders showed a much smaller change from 59.88±17.24 mmHg to 51.25±13.74 mmHg (p<0.001). The pulmonary vascular resistance changed from 910±344 dynes.sec.cm^{-5} to 256±109 dynes.sec.cm^{-5} in the responders whereas it showed a much smaller drop from 663±233 dynes.sec.cm^{-5} to 605±222 dynes.sec.cm^{-5} (p<0.001).

Weak positive linear correlation (r=0.334, p<0.07) between preoperative plasma ET-1 levels and the preoperative pulmonary vascular resistance of subjects was revealed. (Figure-2)

However, the postoperative plasma ET-1 levels and pulmonary vascular resistance of patients showed a highly significant linear correlation between these two variables (r=0.700, p<0.001). (Figure-3)

Table-1: Comparison of plasma Endothelin-1 levels and Hemodynamic Parameters before and after administration of inhaled NO in responders and non-responders

Variables	Responders (n= 22)		Non responders (n= 8)	
	Pre iNO	Post iNO	Pre iNO	Post iNO
Plasma endothelin-1 (fmol/l)	0.627±0.20	0.466±0.088	0.56±0.079	0.68±0.12*
Cardiac output (l/min)	2.99±0.59	4.17±1.14	2.9±0.52	4.3±1.1
Mean pulmonary artery pressure (mmHg)	65.73±14.87	28.09±6.93	59.88±17.24	51.25±13.74*
Pulmonary vascular resistance (dynes.sec. cm^{-5})	910±344	256±109	663±233	605±222*

p<0.001 for the differences in post iNO values between responders and non responders.

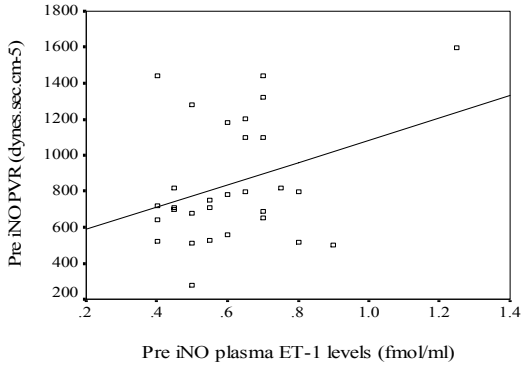

Figure-2: Scattergram of preoperative plasma endothelin-1 levels and pulmonary vascular resistance showing a weak positive linear correlation, with r = 0.334 and p = 0.071. (n=30)

iNO= inhaled nitric oxide; PVR= Pulmonary Vascular Resistance; ET-1= Endothelin-1

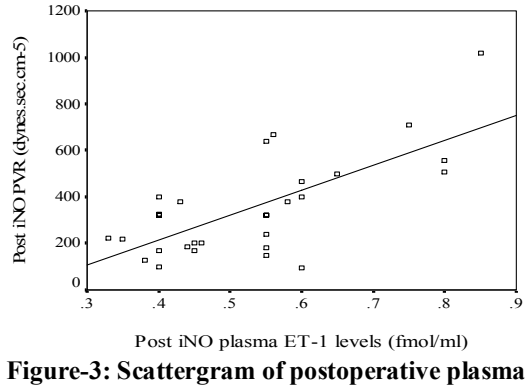

Figure-3: Scattergram of postoperative plasma endothelin-1 levels and pulmonary vascular resistance showing a positive linear correlation with r = 0.700, p<0.001, (n=30)

iNO= inhaled nitric oxide; PVR= Pulmonary Vascular Resistance; ET-1= Endothelin-1.

DISCUSSION

Raised levels of Plasma ET-1 in patients with mitral stenosis are documented in numerous studies.[11–13] Our study also showed high baseline endothelin-1 levels (0.61±0.81 fmol/l vs 0.033 fmol/l) as compared to normal. An interesting finding was that baseline levels of ET-1 were higher (0.627±0.20 fmol/l) in patients who responded well to iNO therapy, as compared to the ones showing poor response.

However, the non-responders showed significant increase in plasma ET-1 levels after nitric oxide administration. On the other hand, in responders the levels of plasma ET-1 dropped drastically after iNO therapy. This difference may be attributed to the difference in the way pulmonary vasculature responds to iNO therapy in different individuals.

In a study done on piglets, it was shown that pulmonary hypertension produced by infusion of ET-1, was not diminished by 60 ppm of inhaled NO therapy unless endogenous production of NO was decreased/ blocked with NO inhibitor L-NAME (L-Nitro-Arginine MethylEster).[14]

In another study, the plasma ET-1 concentration dropped after iNO therapy in patients with secondary PH undergoing Left Ventricular Assist Device (LVAD) Implantation.[15]

Our data support the contention that increased ET-1 secretion maintains pulmonary hypertension that may not be ameliorated by selective pulmonary vasodilators such as iNO in some patients.

Measurement of plasma ET-1 levels was carried out in blood samples drawn from the antecubital vein**,** which is shown to have concentrations of ET-1 same as central veins in patients with MS. [16]

A positive linear correlation is documented in our study between pulmonary vascular resistance and plasma ET-1 levels. Christoph Rubens *et al* in a study carried out in patients with primary pulmonary hypertension, showed a strong correlation between elevated plasma levels of ET-1 and pulmonary vascular resistance, mean pulmonary artery pressure, cardiac output, and cardiac index ($p<0.05$) and suggested that levels of circulating ET-1 might become a prognostic marker and serve as a tool for the selection of patients who may benefit from treatment with ET-receptor antagonists.[17] A positive linear correlation between increased ET-1 levels and mean pulmonary artery pressure($r=0.65$, $p=0.04$) was also documented by Yamamoto and associates[18] and was attributed to response to increased pulmonary artery pressure. However, the only positive correlation found in this study was between pre and post iNO therapy PVR and plasma ET-1 levels. Probably this difference was due to the totally different aetiologies of PH in the study populations.

In another study[19], ET-1 concentration was evaluated as a predictor of postoperative regression of pulmonary hypertension in patients with MS. Due to the time constraint, we could not follow up these patients to observe the regression patterns of plasma ET-1 levels, after relief of obstruction, i.e., mitral valve replacement.

CONCLUSION

In conclusion, the study supports the hypothesis that plasma ET-1 may be a key mediator in maintaining high pulmonary vascular resistance, resulting in a poor response to nitric oxide inhalation after the corrective surgery for mitral stenosis. It also hints that ET-1 blockers can be helpful in the treatment of PH, in patients refractory to treatment with conventional or selective vasodilators.

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