Evaluation of Endothelin-1 and Von Willebrand Factor as Biomarkers of Pulmonary Hypertension in Children with Congenital Heart Disease

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Abstract:

This study was done to delineate the role of endothelin-1 (ET-1) and von Willebrand factor (vWF) in the pathophysiology of pulmonary hypertension (PHT) secondary to congenital heart disease. Forty-three children (29 males, 14 females) with cyanotic and acyanotic congenital heart diseases were enrolled in this study. Their age ranged between 4 months and 5.10 year. Plasma ET-1 levels and vWF:Ag activity were assayed by enzyme linked immunosorbent assay. Enrolled children were divided into three groups according to pulmonary artery pressure (PAP), group I with normal PAP (≤ 30 mmHg) (n=15); group II children with mild PHT (PAP 31-49 mmHg) (n=14); group III with moderate or severe PHT (PAP>50mmHg) (n=14). Twelve perfectly matched healthy children were enrolled as a control group. The results of the present study showed that plasma ET-1 levels in group I were significantly higher than that in control group (P<0.001), on the other hand no significant differences were noted in vWF:Ag% in both groups. Plasma endothelin-1 and vWF:Ag were significantly elevated in all groups with PHT Vs controls (P<0.001& P<0.001). Plasma endothelin-1 and vWF:Ag% were significantly elevated in group III Vs both group II and I (P<0.001). Plasma endothelin-1 and vWF:Ag% were significantly elevated in group II Vs group I (P<0.001& P<0.001). Plasma ET-I levels and vWF:Ag% were positively correlated with pulmonary artery pressure in group II and III (P<0.001& P<0.001).

Conclusions: Elevated ET-I and vWF may contribute directly to development of pulmonary hypertension in children with congenital heart diseases. ET-I and vWF estimation could be used as non-invasive early markers of pulmonary hypertension in such children, particularly in post-operative evaluation. Our data are in keeping with evidence of significant coagulation abnormalities in pulmonary hypertension and the need for chronic anticoagulant therapy may increase survival in children with pH. These facts opened the door for exploring therapeutic anti-ET-1 and anti- vWF agents in the treatment of pulmonary hypertension in children.

Introduction:

Pulmonary arterial hypertension is a serious progressive condition with a poor prognosis if not identified and treated early. Because the symptoms are nonspecific and the physical findings can be subtle, the disease is often diagnosed in its later stage. Advances in technology allow earlier diagnosis based on improved understanding of the vascular biology of the normal and hypertensive pulmonary circulation. Recent evidences suggest that pulmonary vascular endothelium is an important determent of vascular tone. This has led to hypothesis that, the endothelial injury, secondary to congenital heart disease especially with increased pulmonary blood flow, play a role in the development of pulmonary hypertension and its associated increased vascular reactivity. The endothelial cells can elaborate a variety of substances such as endothelin, prostacyclin, von Willebrand factor (vWF), selectins and heparin, which regulate pulmonary blood flow vascular resistance. Endothelin-1 (ET-1) is a peptide produced primarily by vascular endothelial cell, characterized as a powerful vasoconstrictor and mitogen for smooth muscle. An activation of ET-1 system has been demonstrated in plasma of pulmonary hypertensive children with congenital heart disease. Furthermore, there is a strong correlation between ET-1 expression and pulmonary vascular resistance in children with pulmonary arterial hypertension.

Endothelial cell injury is followed by rapid release of von Willebrand factor (vWF) from storage granules into the circulation. For this reason, plasma antigenic activity of vWF has been used widely as a marker of endothelial cell injury in cases of pulmonary hypertension in congenital heart disease. A possibility exists that, the magnitude of endothelial cell dysfunction is correlated with the extent and severity of pulmonary microvascular damage in pulmonary hypertension. If so, the assessment of
endothelial cell function using biochemical markers as endothelin and vWF:Ag, might have diagnostic, prognostic and therapeutic implications.17-19 Both endothelin-1 and vWF:Ag have different origin and may provide different informations about endothelial dysfunction in pulmonary hypertension.20-23

We therefore planned the present study to use these biomarkers by noninvasive methods, as early predictors of progressive pulmonary hypertension (PH) as well as their correlation with endothelial dysfunction in congenital heart disease with and without pulmonary hypertension.

**Subjects and Methods:**

This study was carried out at Cardiology Unit, Pediatric Department, Tanta University Hospital during the period from October 2002 to December 2004. We enrolled 43 consecutive children with congenital heart disease (CHD) affiliated to 29 male and 14 female. Their age ranged between 4 months and 5.10 year. These patients were divided into three groups according to pulmonary artery pressure (PAP): group I with normal pulmonary arterial pressure: PAP ≤ 30 mmHg (n=15), group II with mild PHT (PAP 31-49 mmHg) (n=14), and group III with moderate and severe PHT (PAP ≥ 50 mmHg)(n=14). The control group consisted of 12 age and sex-matched healthy children.

For all children, the following measures were done:
1. Detailed medical history as well as clinical examination.
2. Echocardiographic examination was performed using echocardiographic machine (HP) with 3.5, 5 μHZ transducers. The pulmonary artery pressure was measured from the left parasternal short axis view at the level of the annulus.24

**Blood collection:**

Peripheral blood samples for detection of von Willebrand factor and endothelin-1 were drawn and divided into 2 specimens, the first specimen for (vWF: Ag %) was collected immediately into vacutainer tube containing 3.8 % disodium citrate as anticoagulant (1 citrate + 9 blood), the other specimen was collected into vacutainer tube containing EDTA as anticoagulant (1 mg / ml of blood). These tubes were gently rocked several times and centrifuged immediately for 20 minutes. The plasma were separated and stored at −20 °C until analysis.25

**Biochemical Determinations:**

1. Plasma levels of endothelin-1 were determined by sandwich Enzyme Immuno-Assay (EIA) kit provided by Assay designs, TitreZyme Kits, Cat. No. 900-020. The concentrations were calculated from standard curve.25

2. Plasma levels of von Willebrand factor antigen activity (vWF:Ag %) were determined by sandwich Enzyme Linked Immunosorbent Assay (ELISA) provided by Assaypro, Cat. No. EV2030-1. Results were obtained by comparison with standard curve.26

**Statistical Analysis:**

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. The range, mean and standard deviation were calculated. For comparison between groups, the F value of analysis of variance (ANOVA) was calculated and Scheffe test was performed to compare between each two means if (F) value was significant. Pearson's correlation coefficient (R) was calculated to test the association between two variables. Significance was adopted at P<0.05 for interpretation of results of tests of significance.

**Results:**

**Characteristics of studied groups:**
Tables I and II show characteristics of children with congenital heart defects enrolled in this study.

**Changes in plasma endothelin-1 levels** (tables III & V and figure 1):
Table III shows that:
- Plasma endothelin-1 was significantly elevated in all groups with congenital heart lesions with or without PHT Vs controls (group I = 2.48±0.41, group II = 3.96±0.58, group III = 7.35±1.03 Vs Controls 0.94±0.30 pg/ml) (P = 0.001).
- Plasma endothelin-1 was significantly elevated in group III Vs both group II and I (P<0.001).
- Plasma endothelin-1 was significantly elevated in group II Vs group I (P<0.001).

Table V shows that:
- Plasma ET-I levels were positively correlated with pulmonary artery pressure in groups II and III (r=0.823, P<0.001).

Figure 1 illustrates the significant positive correlations between plasma ET-I levels and the degree of pulmonary hypertension.

**Von Willebrand factor antigen activity changes** (tables IV & V and figure 2):
Table IV shows that:
- Plasma vWF antigen % showed no significant difference between children with CHD without pulmonary hypertension (group I) (111.92±6.44%) and healthy control (89.17±11.67%) (P>0.01).
- Plasma vWF antigen % was significantly elevated in group II and III (164.54±46.9%, 206.77±18.65%), respectively (P<0.001) as compared with healthy control (89.17%±11.67%).
Table V shows that von Willebrand antigen % levels in children with CHD were positively correlated with pulmonary artery pressure (r = 0.673, P < 0.001). Figure 2 illustrates the significant positive correlations between plasma vWF antigen % levels and the degree of pulmonary hypertension.

Table I: Congenital cardiac defects in studied patients

<table>
<thead>
<tr>
<th>Group I (15 cases)</th>
<th>Group II (14 cases)</th>
<th>Group III (14 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyanotic (9 cases)</td>
<td>Cyanotic (6 cases)</td>
<td>Acyanotic (7 cases)</td>
</tr>
<tr>
<td>VSD, bicuspid aortic valve</td>
<td>TGA without PS</td>
<td>TOF</td>
</tr>
<tr>
<td>VSD</td>
<td>TGA without PS</td>
<td>ASD</td>
</tr>
<tr>
<td>Partial AVSD + Left AV valve insufficiency</td>
<td>TGA</td>
<td>ASD, AS</td>
</tr>
</tbody>
</table>


Table II: Characteristics of studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Normal control</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years: Mean ± SD</td>
<td>2.5 ± 2.1</td>
<td>3.5 ± 2.2</td>
<td>3.8 ± 2.9</td>
<td>2.4 ± 1.5</td>
<td>1.200</td>
<td>0.322</td>
</tr>
<tr>
<td>Sex (Male/Female)</td>
<td>9/5</td>
<td>10/5</td>
<td>10/4</td>
<td>8/4</td>
<td>1.390</td>
<td>0.258</td>
</tr>
<tr>
<td>Systemic arterial pressure (mmHg): Mean ± SD</td>
<td>85 ± 13</td>
<td>96 ± 11</td>
<td>96 ± 11</td>
<td>90 ± 14</td>
<td>1.390</td>
<td>0.258</td>
</tr>
<tr>
<td>Heart rate (BPM): Mean ± SD</td>
<td>100 ± 12</td>
<td>107 ± 10</td>
<td>103 ± 11</td>
<td>105 ± 15</td>
<td>0.730</td>
<td>0.542</td>
</tr>
<tr>
<td>PAP (mmHg): Mean ± SD</td>
<td>20 ± 3.29</td>
<td>39.85 ± 9.05</td>
<td>61 ± 17.25</td>
<td>19.42 ± 2.61</td>
<td>194.79</td>
<td>0.001*</td>
</tr>
</tbody>
</table>


Table III: Comparison of plasma endothelin-1(pg/ml) among studied groups

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Range</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.5-1.4</td>
<td>0.94</td>
<td>±0.30</td>
</tr>
<tr>
<td>Group I</td>
<td>1.9-3.2</td>
<td>2.48</td>
<td>±0.41</td>
</tr>
<tr>
<td>Group II</td>
<td>3.1-4.9</td>
<td>3.96</td>
<td>±0.56</td>
</tr>
<tr>
<td>Group III</td>
<td>6.1-10.1</td>
<td>7.35</td>
<td>±1.03</td>
</tr>
<tr>
<td>F, p</td>
<td>223.600, p = 0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheffe test
- Control group significantly different from groups I and III
- Group I significantly lower from II and III
- Group II significantly different from I and III
- Group III significantly different from I and II

*Significant
Table IV: Comparison of von Willebrand antigen (vWF:Ag%) among studied groups

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Range</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>73-108</td>
<td>89.17</td>
<td>±11.67</td>
</tr>
<tr>
<td>Group I</td>
<td>101-122</td>
<td>111.92</td>
<td>±6.44</td>
</tr>
<tr>
<td>Group II</td>
<td>50-210</td>
<td>164.54</td>
<td>±46.90</td>
</tr>
<tr>
<td>Group III</td>
<td>184-246</td>
<td>206.77</td>
<td>±18.65</td>
</tr>
<tr>
<td>F, p</td>
<td>51.070, p=0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Scheffe test
- Control group significantly different from groups II and III
- Group I significantly different from groups II and III
- Group II significantly different from I and III
- Group III significantly different from I and II

*Significant

Table V: Correlation between studied variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pulmonary hypertension</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF:Ag%</td>
<td></td>
<td>0.473</td>
<td>0.001*</td>
</tr>
<tr>
<td>ET-1</td>
<td></td>
<td>0.823</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*Significant

Discussion:

The pathogenesis of pulmonary hypertension (PHT) involves a complex and multifactorial process. Endothelial dysfunction seems to play an integral role in mediating the structural changes in the pulmonary vasculature. Early endothelial damage is associated with enhanced pulmonary vascular reactivity that may contribute to the development of pulmonary hypertension.

In the present study, there was a significant increase of endothelin-1 in children with congenital heart disease without pulmonary hypertension when compared to control children. This is in accordance with the results reported by Jia et al., who accused such elevation to increased pulmonary blood flow independent of pulmonary artery pressure. On other hand, Lopes and others, attributed it, to several agents including hypoxia that induce endothelial cells to secrete endothelin-1 in cyanotic congenital heart disease. However, Gorenflo et al. found that, plasma endothelin-1 concentration did not differ significantly from healthy volunteers compared to children with congenital heart disease.

In the present study, there was a significant increase of endothelin-1 in children with congenital heart disease without different grades of pulmonary hypertension that correlated positively with the severity of pulmonary hypertension, suggesting a possible involvement of endothelin-1 in the pathophysiology of pulmonary hypertension. This is in accordance with the results reported by Collados et al., who stated that, the augmented level of ET-1 was attributed partly from minor increase in local pulmonary release and reduce clearance of this peptide. Song et al. attributed such elevation to increased production of ET-1 in pulmonary circulation, indicating the possible involvement of endothelin-1 in the pathophysiology of pulmonary hypertension, whereas Ruben and others described such elevation as a protective mechanisms and it enhances pulmonary vascular reactivity with pulmonary hypertension. However, Tutar et al. found no correlation between plasma ET-1 levels and pulmonary artery pressure.

According to the results of the present study, plasma vWF:Ag% was significantly increased in children with PHT when compared to children with CHD without PH. The present results agree with Lopes and others, who reported that there is increased level of vWF:Ag in PH. Penny et al. suggested that raised concentration of vWF:Ag in patients with pulmonary hypertension was due to increased endothelial damage in these patients. In the present study, a significant positive correlation was detected between plasma vWF:Ag% and pulmonary arterial pressure (r=0.473, P<0.001). These findings are consistent with previous studies, which showed that vWF:Ag% directly correlated with severity of endothelial damage in PHT. Sakamaki et al. suggested that in pulmonary hypertension, endothelial cells release large amount of vWF into the circulation by increased amount of fibrin, cytokines and thrombin. Also Rabinovitch et al. suggested that, in PHT, the selective loss of large vWF multimer might be related to thrombin generation in vivo, since the high molecular weight forms of vWF interact with platelet in the presence of thrombin.

Our data in congenital heart disease without pulmonary hypertension are consistent with those of Turner et al., who reported that there is no significant increase in plasma level of vWF:Ag in congenital heart disease without PHT. On the other hand Caramuru et al. reported that hypoxia itself in cyanotic congenital heart disease seems to influence...
plasma vWF composition as well possible inducing endothelial release of unprocessed vWF molecule. Lopes et al. found that, not all patients with CHD with PHT have biochemical evidence of advanced endothelial cell damage, the possibility is raised that cells that have a particular phenotype are "switched on" to secrete large amount of vWF under appropriate stimuli and over express other biologically relevant molecules such as endothelin-1 as well, therefore explaining the rapid progression in some patients based on these observation. Also, it is well known that the common features of different forms of PHT include imbalance between coagulation, fibrinolytic systems and between proliferation of the vascular smooth muscle cells and cells of intimae induced by ET-1.

Lastly, the raised levels of vWF and ET-1 may be caused by the same hemodynamic disturbances, although the sites of production in the vascular system seems different. So, the use of these selected markers by non invasive methods to evaluate their expression in children with PHT, could be potentially helpful for predicting the clinical course in children with different forms of pulmonary hypertension and their management either medically and surgically.

**Conclusions:**

1. Elevated ET-1 and vWF may contribute directly to development of pulmonary hypertension in children with congenital heart diseases.
2. ET-1 and vWF estimation could be used as non-invasive early markers of pulmonary hypertension in such children, particularly in post-operative evaluation.
3. Our data are in keeping with evidence of significant coagulation abnormalities in pulmonary hypertension and the need for chronic anticoagulant therapy may increase survival in children with pH.
4. These facts opened the door for exploring therapeutic anti-ET-1 and anti-vWF agents in the treatment of pulmonary hypertension in children.

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