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

Mokhtar Abd El-Fatah, Morad A. Morad, Hesham A. Elserogy and Gihan F. Attia
From the Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Tanta University, Egypt

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.[1-3] Because the symptoms are nonspecific and the physical findings can be subtle, the disease is often diagnosed in its later stage.[4] Advances in technology allow earlier diagnosis based on improved understanding of the vascular biology of the normal and hypertensive pulmonary circulation.[5] Recent evidences suggest that pulmonary vascular endothelium is an important determent of vascular tone.[6] 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.[7] 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.[8,9]

Endothelin-1 (ET-1) is a peptide produced primarily by vascular endothelial cell, characterized as a powerful vasoconstrictor and mitogen for smooth muscle.[10] An activation of ET-1 system has been demonstrated in plasma of pulmonary hypertensive children with congenital heart disease.[11] Furthermore, there is a strong correlation between ET-1 expression and pulmonary vascular resistance in children with pulmonary arterial hypertension.[12]

Endothelial cell injury is followed by rapid release of von Willebrand factor (vWF) from storage granules into the circulation.[13] 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.[14]

A possibility exists that, the magnitude of endothelial cell dysfunction is correlated with the extent and severity of pulmonary microvascular damage in pulmonary hypertension.[15,16] 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 preformed 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 ^{o}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, TiterZyme 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= 6.473, 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

Group I (15 cases)		Group II (14 cases)		Group III (14 cases)	
Acyanotic (9 cases)	Cyanotic (6 cases)	Acyanotic (7 cases)	Cyanotic (7 cases)	Acyanotic (7 cases)	Cyanotic (7 cases)
AS, bicuspid aortic valve	TGA without PS	PDA	DORV+VSD+ coarctation	VSD	DORV+VSD
VSD	TOF	VSD	VSD + ASD + pulmonary vascular disease	ASD+VSD	TOF
VSD	Valvular PS+PFO	Complete AVSD+PDA	TAPVC+ASD	PDA	TGA+VSD+DORV
VSD	TGA without PS	Complete AVSD	TAPVC+ASD	VSD + coarctation of aorta	PDA+APW
ASD	TGA with pulmonary stenosis	VSD+PDA	Truncus arteriosus + VSD	AVSD+PDA	TAPVC+ASD
Partial AVSD + Left AV valve insufficiency	TGA	VSD+PDA+AS+MS	TGA+VSD with PDA	ASD+AS+VSD	Truncus arteriosus
ASD		ASD+PAPVC	DORV + PDA + APW + DSM	ASD	TOF
AS					
ASD					

***VSD**: ventricular septal defect, **ASD**: atrial septal defect, **PDA**: patent ductus arteriosus, **TGA**: transposition of the great arteries*
***PS**: pulmonary stenosis , **TOF**: tetralogy of Fallot, **AVSD**: atrioventricular septal defect, **AS:** aortic stenosis, **MS**: mitral stenosis.*
***PFO**: patent foramen ovale, **DORV**: double outlet right ventricle, **APW**: aorticopulmonry window, **DSM**: discrete subaortic membrane*
***TAPVC**: total anomalous pulmonary venous connection,*

Table II: Characteristics of studied groups

Variables		Group I	Group II	Group III	Normal control	F	p
Age in years:	Mean ± SD	2.5 ±2.1	3.5 ±2.2	3.8 ±2.9	2.4 ±1.5	1.200	0.322
Sex (Male/Female)		9/5	10/5	10/4	8/4		
Systemic arterial pressure*(mmHg):*	Mean ± SD	85 ±20	96 ±13	95 ±11	90 ±14	1.390	0.258
***Hemoglobin** (g/dl):*	Mean ± SD	11.9 ±0.8	12.3 ±0.7	12.7 ±0.9	13.2 ±0.8	5.750	0.002*
Heart rate (BPM):	Mean ± SD	100 ±12	107 ±10	103 ±11	105 ±15	0.730	0.542
PAP(mmHg):	Mean ± SD	20 ±3.29	39.85 ±9.05	61 ±17.25	19.42 ±2.61	194.79	0.001*

*PAP: pulmonary artery pressure ,BPM: beat per minute, *Significant.*

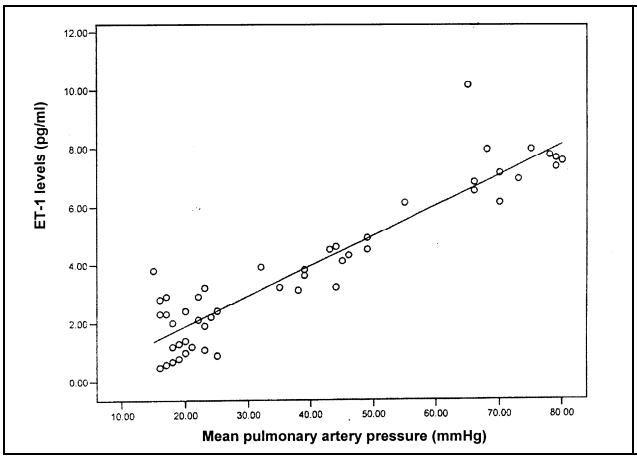

Fig.1: Correlation between ET-1 and mean pulmonary artery pressure

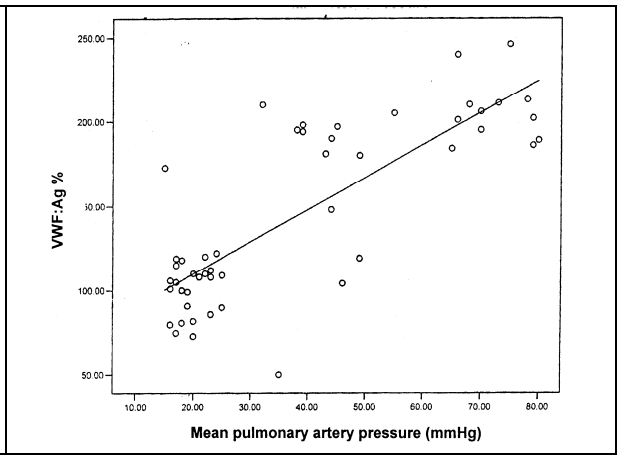

Fig.2: Correlation between vWF:Ag and mean pulmonary artery pressure

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

Studied groups	Range	Mean	S.D.
Normal control	0.5-1.4	0.94	±0.30
Group I	1.9-3.2	2.48	±0.41
Group II	3.1-4.9	3.96	±0.58
Group III	6.1-10.1	7.35	±1.03
F, p	223.600, p = 0.001*		
Scheffe test	• Control group significantly different from groups I,II and III • Group 1 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

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

Variables	Pulmonary hypertension	
	r	p
vWF:Ag%	0.473	0.001*
ET-1	0.823	0.001*

**Significant*

Discussion:

The pathogenesis of pulmonary hypertension (PHT) involves a complex and multifactorial process.[27] Endothelial dysfunction seems to play an integral role in mediating the structural changes in the pulmonary vasculature.[28] Early endothelial damage is associated with enhanced pulmonary vascular reactivity that may contribute to the development of pulmonary hypertension.[29-31]

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.,[27] who accused such elevation to increased pulmonary blood flow independent of pulmonary artery pressure. On other hand, Lopes and others,[4,32] attributed it, to several agents including hypoxia that induce endothelial cells to secrete endothelin-1 in cyanotic congenital heart disease. However, Gorenflo et al.[33] 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 with 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.,[31] who stated that, the augmented level of ET-1 was attributed to partly from minor increase in local pulmonary release and reduce clearance of this peptide. Song et al.[34] 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[35-38] described such elevation as a protective mechanisms and it enhances pulmonary vascular reactivity with pulmonary hypertension. However, Tutar et al.[39] 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,[40,41] who reported that there is increased level of vWF:Ag in PH. Penny et al.[42] 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,[6,9] which showed that vWF:Ag% directly correlated with severity of endothelial damage in PHT. Sakamaki et al.[5] 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.[43] 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.,[14] 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.[44] 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.[40] 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.[13,45] According to these studies chronic anticoagulant therapy has been used in pulmonary hypertension by Cella et al.[10]

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-I and vWF may contribute directly to development of pulmonary hypertension in children with congenital heart diseases.
2. ET-I 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.

References:

1. Beghetti M: Congenital heart disease and pulmonary hypertension. Rev Port Cardiol 2004; 23(2): 273-81.
2. Granton JT and Rabinovitch M: Pulmonary arterial hypertension in congenital heart disease. Cardiol Clin 2002; 20(3); 441-57.
3. Ivy D: Diagnosis and treatment of severe pediatric pulmonary hypertension. Cardiol Rev 2001; 9(4): 227-37.
4. Lopes AA; Maeda NY; Goncalves RC and Bydlowski SP: Endothelial cell dysfunction correlates differentially with survival in primary and secondary pulmonary hypertension. Am Heart J 2000; 139(4): 618-23.
5. Sakamaki F: Coagulation and fibrinolytic abnormality related to endothelial injury in pulmonary arterial hypertension. Nippon Rinsho 2001; 59(6): 1053-8.
6. Subhedar NV: Recent advances in diagnosis and management of pulmonary hypertension in chronic lung disease. Acta Paediatr 2004; 93 (444): 29-32.
7. Tuder RM; Cool CD; Yeager M; Taraseviciene-Stewart L; Bull TM and Voelkel NF: The pathobiology of pulmonary hypertension. Endothelium Clin Chest Med 2001; 22(3): 405-18.
8. Bull TM; Golpon H; Hebbel RP; Solovey A; Cool CD; Tuder RM; Geraci MW and Voelkel NF: Circulating endothelial cells in pulmonary hypertension. Thromb Haemost 2003; 90(4): 698-703.
9. Anderson R; Dart AM; Starr J; Shaw J and Chin-Dusting JP: Plasma C-reactive protein, but not protein S, VCAM-1, von Willebrand factor or P-selectin, is associated with endothelium dysfunction in coronary artery disease. Atherosclerosis 2004; 172(2): 345-51.
10. Cella G; Belotto F; Tona F; Sbarai A; Mazzaro G; Motta G and Fareed J : Plasma markers of endothelial dysfunction in pulmonary hypertension. Chest 2001; 120(4): 1226-30.
11. Chen YF and Oparil S: Endothelin and pulmonary hypertension. J Cardiovasc Pharmacol 2000; 35(4 suppl 2); S49-53.
12. Galie N; Manes A and Branzi A: The endothelin system in pulmonary arterial hypertension. Cardiovasc Res 2004; 1;61(2): 227-37.
13. Ishikawa S; Miyauchi T; Sakai S; Ushinohama H; Sagawa K; Fusazaki N; Kado H; Sunagawa H; Honda S; Ueno H; et al.: Elevated levels of plasma endothelin-1 in young patients with pulmonary hypertension caused by congenital heart disease are decreased after successful surgical repair. J Thorac Cardiovasc Surg 1995; 110 (1): 271-3.
14. Turner Gomes SO; Andrew M; Coles J; Trusler GA; Williams WG and Rabinovitch M: Abnormalities in von Willberand factor and antithrombin III after cardiopulmonary bypass operations for congenital heart disease. J Thorac Cardiovasc Surg 1992; 103(1): 87-97.
15. Goldsmith IR; Blann AD; Patel RL and Lip GY: Plasma fibrinogen, soluble P-selectin, and von Willebrand factor in aortic valve disease: evidence for abnormal haemorheology, platelet activation, and endothelial dysfunction. Heart 2000; 83(5): 577-8.
16. Gorenflo M; Bettendorf M; Brockmeier K and Ulmer HE: Pulmonary vasoreactivity and vasoactive mediators in children with pulmonary hypertension. Z Kardiol 2000; 89(11): 1000-8.
17. Angerio AD and Kot PA: Endothelin-1: possible implications in pulmonary vascular disease. Heart Lung 1997; 26(4): 299-304; quiz 305-6.
18. Huang RJ; Liao CX and Chen DZ: Effect of tetramethylpyrazine on endothelin, von Willebrand factor and thromboxane A2 during cardiopulmonary bypass in patients of congential heart disease with pulmonary hypertension. Zhongguo Zhong Xi YI Jie He Za Zhi 2003; 23(4): 268-71.

19. Friedman R; Mears JG and Barst RJ: Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. Circulation 1997; 4; 96(9): 2782-4.
20. Galatius S; Wroblewski H; Sorensen VB; Bie P; Parving HH and Kastrup J: Endothelin and von Willebrand factor as parameters of endothelial function in idiopathic dilated cardiomyopathy: different stimuli for release before and after heart transplantation? Am Heart J 1999; 137(3): 549-54.
21. Staniloae C; Dupuis J; White M; Gosselin G; Dyrda I and Bois M: Reduced pulmonary clearance of endothelin in congestive heart failure: A marker of secondary pulmonary hypertension J Card Fail 2004; 10(5): 427-32.
22. Tang YJ; Chen LM and Chen Q: Changes of NO and ET-1 in the blood of patients with chronic pulmonary heart disease. Hunan Yi Ke Da Xue Xue Bao 2003; 28;28(1): 59-61.
23. 23. Zhang S; Qi S; Shen X; Zhou S and Jin S: The changes of plasma endothelin concentration and its clinical significance in pulmonary hypertension associated with congenital heart defects . Hunan Yi Ke Da Xue Xue Bao 1997; 22(5): 425-7.
24. Henry WL; DeMaria A; Graisk R; King DL; Kisslo JA and Popp RL: Report of the American Society of Echocardiography on nomenclature and standards in two-dimensional echocardiography. Circulation 1980; 62:212.
25. Chen M; Wu C; Yip HK; Chang HW; Chen CJ; Yu TH and Hung WC: Increased circulating endothelin-1 in rheumatic mitral stenosis: irrelevance to left atrial and pulmonary artery pressures. Chest 2004; 125(2): 390-6.
26. Konkle BA: Laboratory evaluation of von Willebrand disease. Clin Chem 1995,41: 489 – 90.
27. Jia B; Zhang S; Chen Z; Li Z; Li X; Hui W and Ye M: Plasma endothelin-1 concentrations in children with congenital heart defects. Minerva Pediatr 1998; 50(4): 99-103.
28. Kumar P; Kazzi NJ and Shankaran S: Plasma immunoreactive endothelin-1 concentrations in infants with persistent pulmonary hypertension of the newborn. Am J Perinatol 1996; 13(6): 335-41.
29. Levin E, Wu J, Devine DV, Alexander J, Reichart C, Sett S and Seear M: Hemostatic parameters and platelet activation marker expression in cyanotic and acyanotic pediatric patients undergoing cardiac surgery in the presence of tranexamic acid. Thromb Haemost 2000; 83(1): 54-9.
30. Adatia I and Haworth SG: Circulating endothelin in children with congenital heart disease. Br Heart J 1993; 69(3): 233-6.
31. Collados MT; Velazquez B; Borbolla JR; Sandoval J; Masso F; Montano LF and Guarner V: Endothelin-1 and functional tissue factor: a possible relationship with severity in primary pulmonary hypertension. Heart Vessels 2003; 18(1): 12-7.
32. Lopes AA; Caramuru LH and Maeda NY: Endothelial dysfunction associated with chronic intravascular coagulation in secondary pulmonary hypertension. Clin Appl Thromb Hemost 2002; 8(4): 353-8.
33. Gorenflo M, Gross P, Bodey A, Schmitz L, Brockmeier K, Berger F and Bein G, Lange PE: Plasma endothelin-1 in patients with left to right shunt. Am Heart J 1996; 132(6): 1317-9.
34. Song FL; Luo HH and Liu F: Perioperative observation of plasma endothelin in patients with congenital heart disease with pulmonary hypertension underwent open heart surgery. Hunan Yi Ke Da Xue Xue Bao 2001; 28;26(4): 379-80.
35. Rubens C; Ewert R; Halank M; Wensel R; Orzechowski HD; Schultheiss HP and Hoeffken G: Big endothelin-1 and endothelin-1 plasma levels are correlated with the severity of primary pulmonary hypertension. Chest 2001; 120(5):1562-9.
36. Vincent JA; Ross RD; Kassab J; Hsu JM and Pinsky WW: Relation of elevated plasma endothelin in congenital heart disease to increased pulmonary blood flow. Am J Cardiol 1993; 15; 71(13): 1204-7.
37. Zhang ZW; Lin R; Li JH; Hu J and Wang X: Perioperative changes in plasma endothelin and calcitonin gene-related peptide in congenital heart disease with pulmonary hypertension. Zhejiang Da Xue Xue Bao Yi, Xue Ban 2003; 32(3): 212-4.
38. Huang CH, Huang HH, Chen TL and Wang MJ. Perioperative changes of plasma endothelin-1 concentrations in patients undergoing cardiac valve surgery. Anaesth Intensive Care 1996; 24(3): 342-7.
39. Tutar HE; Imamoglu A; Atalay S; Gumus H and Akar N: Plasma endothelin-1 levels in patients with left-to-right shunt with or without pulmonary hypertension. Int J Cardiol 1999; 170(1): 57-62.
40. Lopes AA; Maeda NY and Bydlowski SP: Abnormalities in circulating von Willebrand factor and survival in pulmonary hypertension. Am J Med 1998; 105(1): 21-6.
41. Gill JC, Wilson AD, Endres-Brooks J and Montgomery RR: Loss of the largest von Willebrand factor multimers from the plasma of patients with congential cardiac defects. Blood 1986; 67(3): 758-61.
42. Penny WF; Weinstein M; Salzman EW and Ware JA: Correlation of circulating von Willebrand factor levels with cardiovascular hemodynamics. Circulation 1991; 83(5): 1630-6.
43. Rabinovitch M; Andrew M; Thom H; Trusler GA; Williams WG; Rowe RD and Olley PM: Abnormal endothelial factor VIII associated with pulmonary hypertension and congenital heart defects. Circulation 1987; 76(5): 1043-52.
44. Caramuru LH, Rosangela De P, Soares Rde P, Maeda NY and Lopes AA: Hypoxia and altered platelet behavior influence von Willebrand factor multimeric composition in secondary pulmonary hypertension. Clin Appl Thrombosis/Hemostasis 2003; 9(3): 251-258.
45. Michel RP; Langleben D and Dupuis J: The endothelin system in pulmonary hypertension. Can J Physiol Pharmacol 2003; 81 (6): 542-54.