




Evaluation of preterm infants having bronchopulmonary dysplasia with echocardiography and serum biomarkers

Original Article

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Abstract

Background and objectives: Pulmonary hypertension is frequent in infants with bronchopulmonary dysplasia. Echocardiography is easy to perform, non-invasive, and recommended by guidelines even though solely it is not enough. Catheterisation is gold standard but invasive, expensive, and not cost effective. Therefore, we aimed to assess to find out the role of biomarkers besides echocardiography in the diagnosis of pulmonary hypertension in preterm with bronchopulmonary dysplasia. **Methods:** This study is done during the time period January 2016–2017. The diagnosis of pulmonary hypertension was assessed by echocardiography at 36 weeks later repeated at 3rd and 6th months. We also repeated biomarkers at 3rd and 6th months. The infants born \leq 28 weeks in Erciyes University hospital who were diagnosed bronchopulmonary dysplasia were included. Infants with genetic syndromes, structural lung, and CHDs were excluded. Patients without bronchopulmonary dysplasia but having pulmonary hypertension due to other reasons and patients having echocardiograms without adequate images were excluded. **Results:** At initial, 21/59 patients had bronchopulmonary dysplasia-pulmonary hypertension (Group 1), 21/59 had no bronchopulmonary dysplasia-pulmonary hypertension (Group 2), and 17/59 had bronchopulmonary dysplasia without pulmonary hypertension (Group 3). Systolic pulmonary artery pressure and pulmonary vascular resistance were found high in Group 1 (36 mmHg; $p < 0.001$, 1.25 Woods Unit; $p < 0.0017$, respectively). Tricuspid annular plane systolic excursion values of Group 1 were low. Median serum kallistatin levels of Group 1 were lower than the other groups (230.5 (114.5–300.5) $\mu\text{g/ml}$; $p < 0.005$). During the study period, pulmonary hypertension of 14/21 bronchopulmonary dysplasia-pulmonary hypertension resolved, six patients in Group 3 developed pulmonary hypertension. However, there was no difference in the biomarkers of these six patients. **Conclusion:** In the diagnosis and the follow-up of pulmonary hypertension in bronchopulmonary dysplasia patients, besides echocardiography kallistatin, gelsolin, NT-probrain natriuretic peptide, homocysteine, and cystatin-C levels can be used. Further studies were required with large sample sizes.

Bronchopulmonary dysplasia is one of the most important problems of preterm babies. Besides prematurity, long-term ventilator support and other factors contribute to abnormal pulmonary vascular remodelling leading to pulmonary hypertension, which worsens the clinical course of preterm babies and increases the duration of hospitalisation, mortality, and morbidity. The incidence of pulmonary hypertension in infants with bronchopulmonary dysplasia ranges from 17% to 37% and it has a long-term course that can be clinically evident and underdiagnosed at first but symptomatic at the following time.^{1,2} The diagnosis of pulmonary hypertension is done by catheterisation; however, echocardiography is easy to perform, non-invasive, and recommended by American Heart Association and Pulmonary Hypertension Network. Cardiac catheterisation remains unequivocally the gold-standard diagnostic modality for pulmonary hypertension, but is a high-risk procedure, especially in preterm infants with lung disease. As such, there is an increasing interest in using echocardiography to assist in the diagnosis. In this study, we aimed to assess the effect of pulmonary hypertension in preterm babies with bronchopulmonary dysplasia in terms of echocardiographic variables and serum biomarkers. Serum biomarkers are always popular in the diagnosis and the follow-up of pulmonary hypertensive patients. Serum kallistatin, NT-probrain natriuretic peptide, gelsolin, homocysteine, and cystatin C levels were studied as a biomarker. Kallistatin, a serine proteinase inhibitor, exerts its effect by vascular repair, angiogenesis inhibition, strong vasodilation, inhibition of vascular endothelial growth factor, anti-inflammation, and anti-apoptosis.

We believe that using serum biomarkers and echocardiography together helps in the pulmonary hypertension diagnosis especially in the cases where catheterisation is too risky to perform.

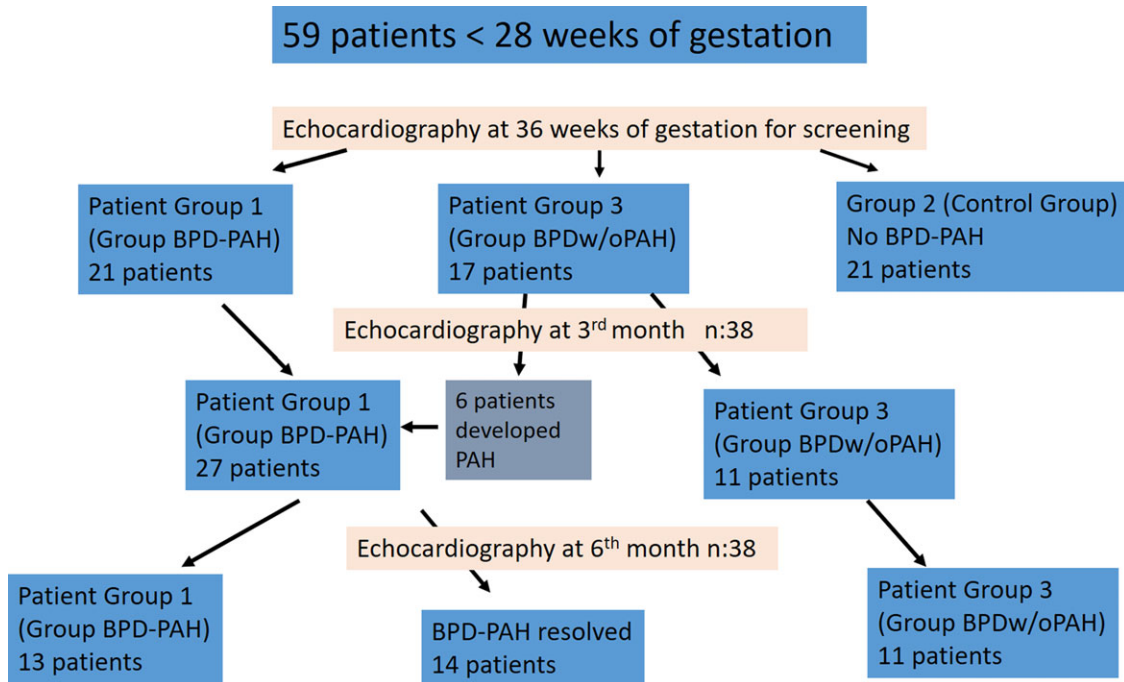


Figure 1. Flow diagram showing the patient selection and intergroup distribution.

Methods

Patients

This prospective study included the infants admitted to our university hospital between January 2016 and January 2017. The inclusion criteria were as follows. The infants who were born ≤ 28 weeks in Erciyes University hospital between the dates: January 2016 and January 2017. Patients with bronchopulmonary dysplasia whose diagnosis was made by the same neonatologist. The diagnosis of bronchopulmonary dysplasia was done by the same neonatologist. Previously, American National Institutes of Health in 2001 guidelines were used.^{3,4} But this rely mostly on the level and duration of oxygen therapy and the clinicians decided that it did not reflect neonatal care and predict long-term morbidity. Therefore, several refinements were done.⁵ Recently, diagnosis based on severity according to the mode of respiratory support administered at 36 weeks' post-menstrual age, regardless of supplemental oxygen used.⁶ Patients without bronchopulmonary dysplasia and without pulmonary hypertension were included. Patients without structural lung, CHD (except patent foramen ovale), and genetic syndromes were included.

Infants with genetic syndromes, structural lung, and CHDs except patent foramen ovale were excluded from the study. Patients without bronchopulmonary dysplasia but having pulmonary hypertension due to other reasons were excluded. The patients having echocardiograms without adequate images to assess tricuspid regurgitation and tricuspid annular plane systolic excursion in order to estimate pulmonary pressure were excluded. If patent ductus arteriosus persists or becomes evident lately and the cardiologist thought that it had a contribution to the pulmonary hypertension (haemodynamically significant), these patients were excluded. Patient selection was shown in the flow diagram (Fig. 1)

Demographic data of patients' birth weight, gender, and neonatal morbidity like surfactant administration, presence of patent ductus arteriosus, if patent ductus arteriosus is present

which treatment used, duration of mechanical ventilation, diuretic usage, intraventricular haemorrhage and its grade, necrotising enterocolitis and its severity, any surgical procedures and late-onset sepsis were recorded. Moreover, maternal and perinatal risk factors like the presence of chorioamnionitis, maternal hypertension, and administration of antenatal steroids were also recorded.

Echocardiography

Echocardiographic measurements were performed with a General Electrics Vivid-7 pro equipped with a 3–7 MHz for 2-dimensional and colour flow Doppler mapping by a single blinded paediatric cardiologist. Investigations were done in parasternal long, short axis, four chamber, five chamber views using M mode and Doppler wave. Two-dimensional measurements were done according to the guidelines of American Society of Echocardiography. Ejection fraction and end diastolic or end systolic volumes were calculated using the Teicholtz method. Estimated systolic pulmonary artery pressure can be determined by using the modified Bernoulli equation: 4 Velocity^2 , where Velocity is the maximum velocity of the tricuspid valve regurgitation jet, measured by continuous wave Doppler, added to the estimated right atrial pressure. Tricuspid valve regurgitation, systolic pulmonary hypertension, pulmonary acceleration time, pulmonary velocity time integral, fraction of area changes of right ventricle, right ventricle myocardial performance index, right ventricle fraction of shortening, tricuspid annular plane systolic excursion, left ventricle eccentricity index, and left ventricle myocardial performance index parameters were measured by echocardiography.⁷ PVR can be estimated by the formula: $\text{PVR (Woodunits)} = 10 \times (\text{tricuspid valve regurgitation} / \text{Velocity Time Index}_{\text{Right Ventricle Outflow Tract}}) + 0.16$.⁸ Left ventricle eccentricity index calculated by the formula (eccentricity index, $=D2/D1$); where D1 is the ventricular diameter perpendicular to the interventricular septum bisecting D2: the diameter parallel to interventricular septum.⁹ Inferior vena cava distensibility index is

calculated by measuring the D_{\max} and D_{\min} of inferior vena cava from the subcostal view, inferior vena cava distensibility index $=D_{\max}-D_{\min}/D_{\min}$.¹⁰ First echocardiograms were performed at 36 weeks for screening later repeated at 3rd and 6th months after the first echocardiogram. Diagnosis of pulmonary hypertension was done according to the presence of at least one of the following criteria: (1) tricuspid valve regurgitation $\geq 3\text{m/s}$ in the absence of pulmonary stenosis or (2) estimated right ventricular systolic pressure > 40 mmHg, or right ventricular systolic pressure/systemic systolic blood pressure > 0.5 , (3) cardiac shunt with bidirectional or right-to-left flow, (4) flat or left-deviated interventricular septum configuration and right ventricular systolic pressure hypertrophy with chamber dilation.¹¹ Infants with evidence of CHD or structural lung and airway malformations were excluded.

Serum biomarkers

Serum biomarkers: kallistatin, NT-probrain natriuretic peptide, gelsolin, homocysteine, and cystatin C levels were measured in the beginning of the study when the patients at postmenstrual age 36 weeks and repeated at 3rd and 6th months.

Serum kallistatin levels

Blood for kallistatin level was taken to the tubes centrifuged at 4000 r.p.m. for 10 min at 4°C. Kallistatin levels were determined by ELISA (R&D Systems, Inc. Minneapolis, USA) as described previously.¹²

Serum NT-probrain natriuretic peptide levels

Blood samples were centrifuged at +4°C and 1500 rpm for 5 min. Samples were studied with ELISA method using Biomedica N-terminal Probrain natriuretic peptide commercial kits (NT-Probrain natriuretic peptide enzyme immunoassay kit Biomedica, Bratislava, Slovakia) and Elecsys® 1010 autoanalyzer (Roche Diagnostics, Basel, Switzerland).

Serum gelsolin levels

The blood samples were immediately placed in sterile ethylene diamine tetra acetic acid test tubes and centrifuged at 2000–3000 r.p.m. for 20 min at 4°C to collect plasma. The concentration of gelsolin in plasma was analysed by enzyme-linked immunosorbent assay using commercial kits (Hangzhou Eastbiopharm Co., Ltd, Hangzhou, Zhejiang, China) in accordance with the manufacturer's instructions.

Serum homocysteine levels

Plasma homocysteine was measured using an auto-biochemical analyzer (AU5800, Beckman Coulter Company, USA) by the enzymatic method. The detection reagents were provided by DiaSys Diagnosis System GmbH (Shanghai) Co., Ltd.

Serum cystatin-c levels

Cystatin C levels were determined with an immune nephelometry method on a ProSpec analyzer (BN ProSpec, Siemens, Frimley, UK) using latex-enhanced particles coated with anti-cystatin C antibodies.¹³

The study was approved by the local research Ethics Committee (Erciyes University Ethical Committee 2015/492). All the parents were informed that written consent was taken from each of them about participating in the study. The study protocol conforms to

the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee. The study was also registered to Clinical Trials. Clinical Trials. Gov Identifier: NCT05082272

Statistical analysis

Data analysis was performed using IBM SPSS Statistics Standard Concurrent User V 25 (IBM Corp, Armonk, New York, USA). Continuous variables were expressed either as mean \pm standard deviation or median 25th–75th percentiles. Distribution of numerical data is evaluated by Shapiro–Wilk normality test Q–Q graphics. Categorical variables were expressed as counts, proportions, and percentages. Kruskal–Wallis test was used to determine whether or not there is a statistically significant difference between the medians of three or more independent groups with non-parametric values. If statistical significance was detected ($p < 0.05$), post-hoc analysis–Tukey test was performed for pairwise comparisons. For the repeated measures analysis of a dependent group, Friedman test was used.

Results

All infants included in the study were divided into two groups: 38 patients with bronchopulmonary dysplasia and 21 patients without bronchopulmonary dysplasia (control group). In bronchopulmonary dysplasia group, 21 patients had pulmonary hypertension and 17 did not have pulmonary hypertension shown in the flow diagram (Fig. 1). The prevalence of pulmonary hypertension was found in our study as 35.5%. Infants in Group 1, compared to Group 3 infants, had lower birth weight (Table 1). The number of respiratory infections in bronchopulmonary dysplasia groups was significantly higher than the other groups ($p : 0.001$).

Furthermore, systolic pulmonary artery pressure and pulmonary vascular resistance values were found significantly high in Group 1. Perinatal, natal, and postnatal risk factors for bronchopulmonary dysplasia and pulmonary hypertension like birth weight, gender, multiple gestation, prenatal steroid usage, presence of preeclampsia, choiroamnionitis, having caesarean section early membrane rupture, intrauterine growth retardation, intubation at birth, mechanical ventilation in first 24 hours, retinopathy of prematures, necrotising enterocolitis, intraventricular haemorrhage, and patent ductus arteriosus closure treatment were investigated. In our study, we did not find a correlation between pulmonary hypertension and maternal perinatal risk factors.

Duration of respiratory support

The percentage of Group 1 patients who were intubated at birth was 61.9%. Median duration of hospital stay was 109 (83–265) days. The 47.6% of patients required home oxygen supply. The mechanical ventilation support duration of each group was compared and no statistical significance was detected between Group 1 and Group 3. Most of the patients of the control group did not have mechanical ventilation support; therefore, statistical significance was detected when compared with this group with the others ($p : 0.029$, $p : 0.002$, Table 1). When the groups were compared in terms of oxygen support, statistical significance was only detected between Group 1 with Group 2 (control group) ($p : 0.002$).

Table 1. Epidemiologic features, echocardiographic, and serum biomarkers of groups. All the values were measured baseline

	BPDwPHT+ Group 1	BPDw/oPHT Group 3	Control Group (w/oBPD): Group 2	P value
Number of patients	21	17	21	
Birth Weight (gr)	1000(850–1340)	1100(832–1345)	1360(1100–1512)	P:0.051
Mechanical ventilation support (days)	1.0 (0.00–23.0)	23.0 (3.0–29.5)	0.0 (0.0–3.0)	P ^b < 0.001
Oxygen support (days)	14.0 (3.5–24.0)	1.0 (0.0–23.0)	3.0 (2.0–5.0)	P ^c :0.013
CPAP (days)	4.0 (1.5–8.0)	1.0 (0.0–10.0)	0.0 (0.0–3.0)	P:0.931
Systolic PAP (mmHg)	36 (30.4–40.7)	21 (17.8–23.4)	21.2 (18.0–28.4)	P ^d < 0.001
PVR(Wood units)***	1.25 (1.12–1.82)	0.93 (0.86–1.23)	1.01 (0.81–1.29)	P ^e :0.017
TAPSE	12.0 (11.25–14.35)	12.75 (11.25–15.37)	13.5 (12.25–14.25)	0.406
LV eccentricity index	1.17 (1.09–1.49)	1.12 (1.06–1.27)	1.14 (1.07–1.36).	0.234
LV TEI	0.8 (0.63–0.89)	0.69 (0.52–0.83)	0.66 (0.47–0.82)	0.118
RV TEI	0.75 (0.60–0.87)	0.88 (0.54–0.94)	0.63 (0.52–0.76)	0.076
FAC of RV	0.51 (0.34–0.56)	0.3 (0.21–0.43)	0.45(0.24–0.55).	0.055
IVC Index	0.14 (0.05–0.22)	0.1 (0.07–0.23)	0.18 (0.11–0.26)	0.496
Gelsolin (mg/L)	210 (120.0–382.5)	165.0 (113.0–392.5)	388.0 (155.0–407.5)	0.081
Kallistatin (µg/ml)	230.5 (114.5–300.5)	259.0 (152.0–325.5)	357.0 (265.0–416.5)	P ^f :0.007
ProBNP (pg/ml)	810.5 (483.5–1496.5)	1479.0 (772.4–2904.2)	1170.0 (912.4–1689.0)	0.111
Homocystein (µmol/L)	9.2 (6.4–11.1)	9.8 (8.7–12.8)	9.6 (6.55–15.3)	0.576
Cystatin C (mg/L)	1.73 (1.48–1.88)	1.64 (1.52–1.83)	1.74 (1.62–1.86)	0.542

The values shown in this table are baseline measurements. Continuous variables were expressed either as mean ± standard deviation or median 25th–75th percentiles

BPD = bronchopulmonary dysplasia; CPAP = continuous positive airway pressure; FAC = fractional area change; IVC = inferior vena cava; LV = left ventricle; PHT = pulmonary hypertension; PAP = pulmonary arterial pressure; PVR = pulmonary vascular resistance; TAPSE = tricuspid annular plane systolic excursion; TEI = index for myocardial performance.

^aPairwise comparisons were as follows: when Group1 compared with Group2: p : 0.89, when Group1 compared with Group3 p value: 0.002, when Group2 compared with Group3 p value : : 0.018.

^bPairwise comparisons were as follows: when Group1 compared with Group2: p : 0.612, when Group1 compared with Group3 p value: 0.029, when Group2 compared with Group3 p value : : 0.002.

^cPairwise comparisons were as follows: when Group1 compared with Group2: p : 0.746, when Group1 compared with Group3 p value: 0.002, when Group2 compared with Group3 p value : : 0.284.

^dPairwise comparisons were as follows: when Group1 compared with Group2: p < 0.001, when Group1 compared with Group3 p < 0.001, when Group2 compared with Group3 p value : : 0.465.

^ePairwise comparisons were as follows: when Group1 compared with Group2: p:0.008, when Group1 compared with Group3 p:0.027, when Group2 compared with Group3 p: 0.957.

^fPairwise comparisons were as follows: when Group1 compared with Group2: p:0.846, when Group1 compared with Group3 p:0.005, when Group2 compared with Group3 p: 0.069.

Echocardiography results

Echocardiographic measurements were first done in the beginning of study and repeated at 3rd and 6th months. Tricuspid annular plane systolic excursion values of Group 1 were lower than the others at the first examination but not statistically significant. However, in the following exams, tricuspid annular plane systolic excursion values of Group 1 were significantly lower than the others. There was no statistical significance between Group 3 and control group in any tricuspid annular plane systolic excursion values at any time (Table 1). However, when we compared tricuspid annular plane systolic excursion values at the beginning of study and 3rd, 6th months values in each group; statistical significance was seen in Group 1. While pulmonary hypertension was high at the beginning tricuspid annular plane systolic excursion was lower and after treatment, pulmonary hypertension resolved and tricuspid annular plane systolic excursion got higher (Table 1).

Estimated systolic pulmonary artery pressure and pulmonary vascular resistance were found significantly high in Group 1 (36 mm Hg; p < 0.001, 1.25 Woods Unit; p < 0.0017, respectively). Left ventricle eccentricity index, left ventricle myocardial performance index values, right ventricle myocardial performance index, inferior vena cava index, and fraction of area change of right ventricle values of each group were studied.

However, no statistically significant difference was detected within the same group or between the groups (Table 1).

At end of 6th month, pulmonary hypertension of 14 patients in Group 1 resolved, and 6 patients in Group 3 developed pulmonary hypertension.

Serum biomarkers' results

We have measured the serum levels of biomarkers at initial diagnosis and repeated at 3rd and 6th months. Gelsolin levels of groups with bronchopulmonary dysplasia were lower than groups without bronchopulmonary dysplasia but not statistically significant. Group 1 median serum gelsolin levels 210.0 (120.0–382.5) mg/L, Group 2 (Control group) median serum gelsolin levels was 388.0 (155.0–407.5). Group 3 median serum gelsolin levels 165.0 (113.0–392.5) mg/L (Table 1). There was no statistical difference when the levels of gelsolin in 3rd and 6th months were compared with each other. Furthermore, no significance was noted between each group in 3rd and 6th months control (Fig. 2a).

Initial kallistatin was determined as kallistatin 0. Median value of kallistatin 0 of Group 1 was 230.5 (114.5–300.5) µg/ml, 259.0 (152.0–325.5) µg/ml, and 357.0 (265.0–416.5) µg/ml for in Group 3 and Group 2, respectively. When groups were compared, a statistical significant difference was detected between Group 1 with

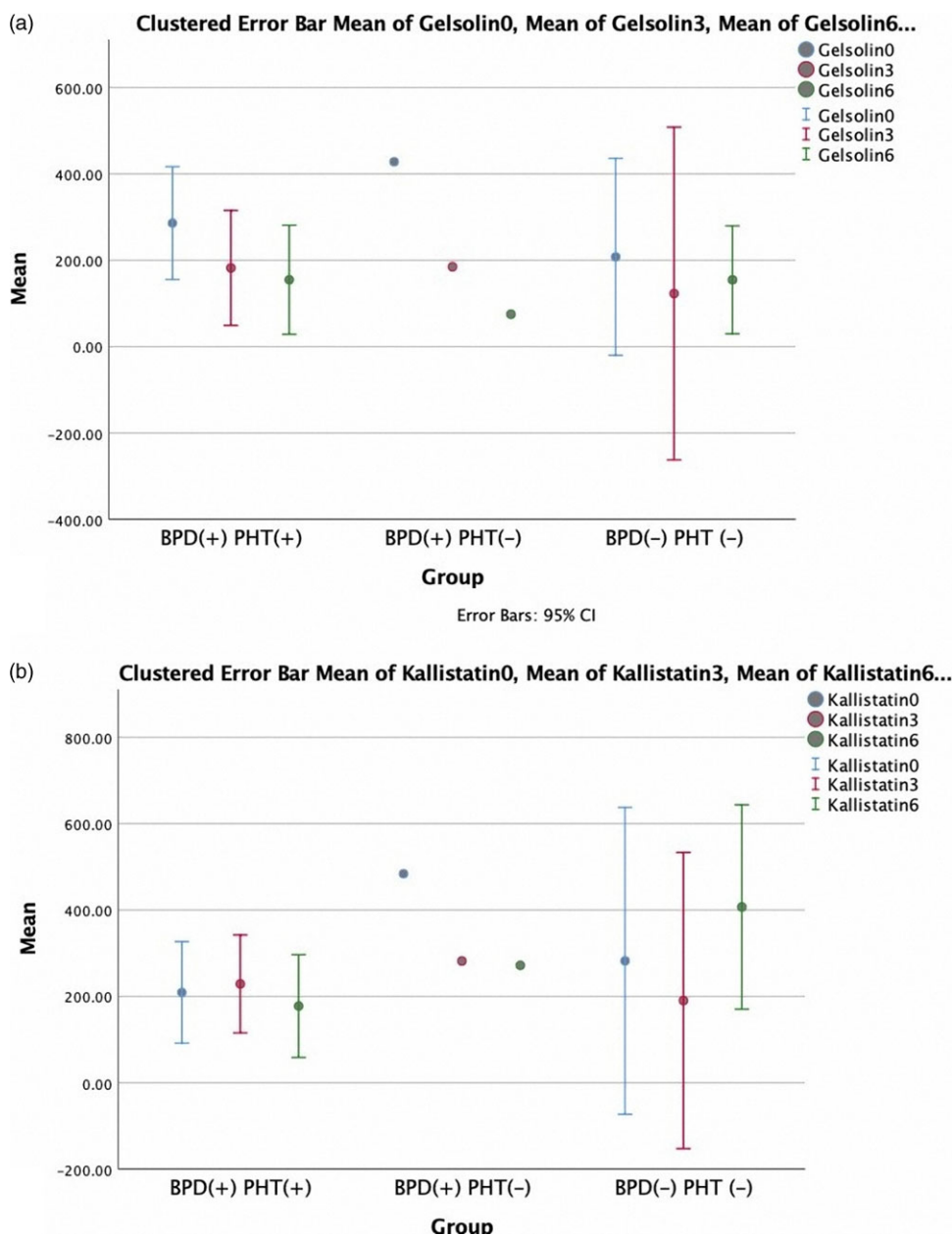


Figure 2. **a:** Cluster error bar of serum gelsolin at initial, 3rd and 6th months. **b:** cluster error bar of serum kallistatin at initial, 3rd and 6th months.

Group 2 as $p : 0.005$ (Table 1). This significant difference was valid at 3rd and 6th months control (Fig. 2b).

Median probrain natriuretic peptide values of Group 1 were 810.5 (483.5–1496.5), Group 3 1479.0 (772.4–2904.2) pg/ml, and Group 2 were 1170.0 (912.4–1689.0) pg/ml. No significant difference was detected between the groups in each control (Figure3A).

Initially, median serum homocysteine levels were lower 9.2 (6.4–11.1) $\mu\text{mol/L}$ in Group 1, when compared with the other groups: Group 3: 9.8 (8.7–12.8) $\mu\text{mol/L}$ and Group 2: 9.6 (6.55–15.3) $\mu\text{mol/L}$ (Table 1). There was no significant difference at 3rd and 6th months in each group and between groups (Fig. 3b).

At beginning, median cystatin C levels of Group 1, Group 3, and Group 2 were 1.73 (1.48–1.88) mg/L, 1.64 (1.52–1.83) mg/L, and 1.74 (1.62–1.86) mg/L. There was no statistical difference between the groups (Table 1). In addition, no significance was

detected in 3rd and 6th months of control in the groups even between the groups.

Discussion

Prematurity is one of the main but not only risk factor for pulmonary hypertension in the patients with bronchopulmonary dysplasia. Long-term ventilator support and other comorbidities (necrotising enterocolitis, intraventricular haemorrhage etc) contribute to pulmonary hypertension. Weismann et al. reported that necrotising enterocolitis, severe intraventricular hemorrhage, and the presence of an atrial septal defect were independent risk factors associated with pulmonary hypertension. They defined that in the absence of bronchopulmonary dysplasia, necrotising enterocolitis was the only identifiable factor associated with pulmonary hypertension.¹⁴ In our study, we could not find a relation between

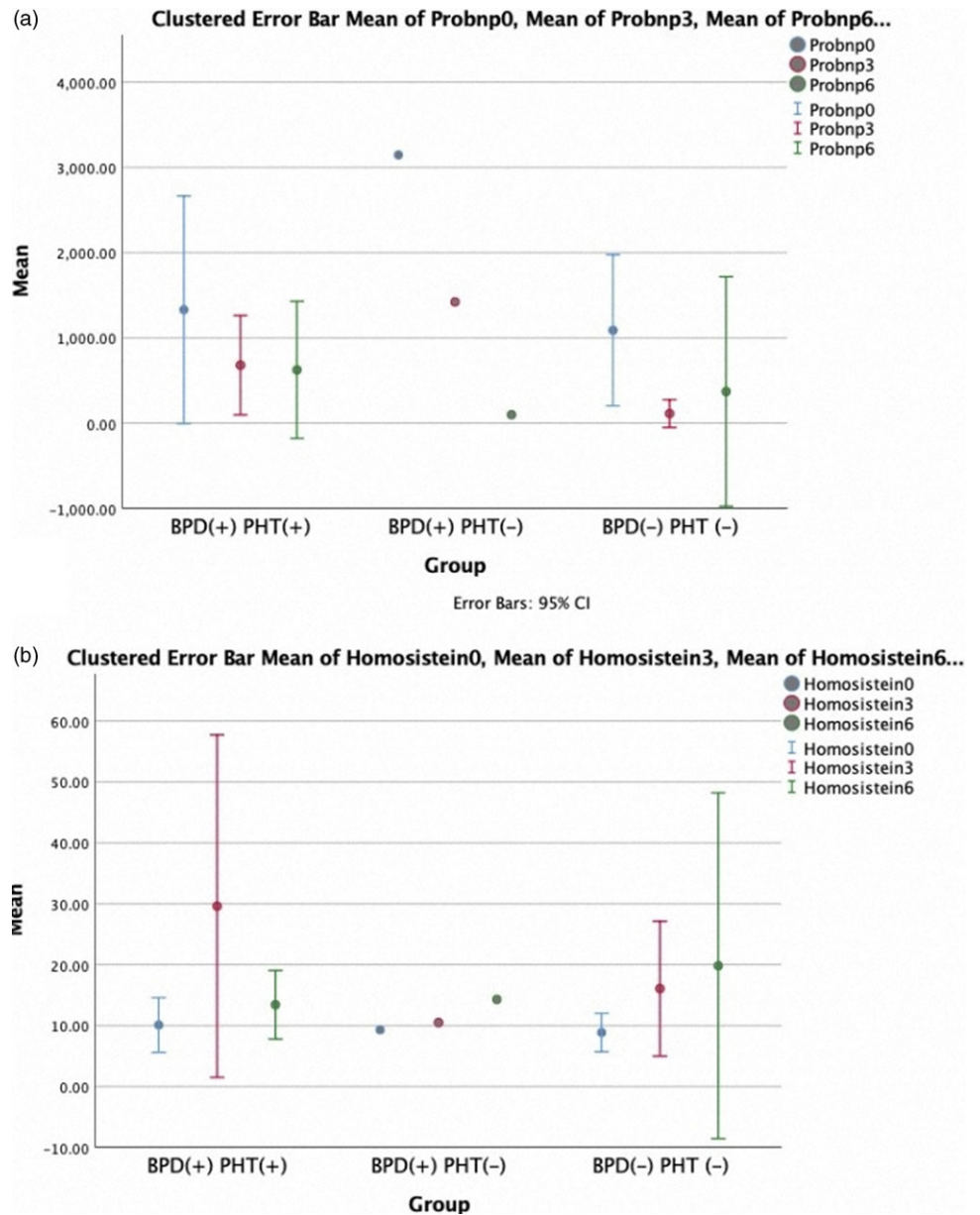


Figure 3. **a:** cluster error bar of serum proBNP at initial, 3rd and 6th months. **b:** cluster error bar of serum homocystein at initial, 3rd and 6th months.

the necrotising enterocolitis and pulmonary hypertension. Furthermore, Vayaltrikkovil et al¹⁵ found that identified ventilator-associated pneumonia was an independent risk factor for pulmonary hypertension. That data are consistent with our study, but we did not specify these infections to subgroups, e.g. ventilator-associated pneumonia.

New guidelines recommend screening by echocardiography at 36 weeks post-menstrual age in premature newborns with moderate to severe bronchopulmonary dysplasia. The prevalence of pulmonary hypertension in our study is 35.5% which is approximately the same reported in previous studies.¹⁶ Standard method for the diagnosis of pulmonary hypertension by echocardiography is estimating right ventricular systolic pressure using tricuspid valve regurgitation. However, there are some limitations in this method like tricuspid valve regurgitation is not overt in every patient. The reason for it can be lung hyperinflation and heart rotation in bronchopulmonary dysplasia patients. Tricuspid regurgitation jet is not the only marker that we use in

the diagnosis of pulmonary hypertension by echocardiography. Differences in eccentricity index and systolic–diastolic ratio were studied by McCrary et al.¹⁷ They did not find any differences between the control and bronchopulmonary dysplasia cohort but eccentricity index was found significantly high in pulmonary hypertension group when compared with Group 3. In our study, left ventricle eccentricity index was also high in Group 1 but not statistically different. This could be due to small sample size.

Inferior vena cava, collapsibility, and distensibility indices are used for the estimation of right atrium pressure and pulmonary hypertension. Actually, obtaining these measurements is really hard for small children while they cannot cooperate for ventilation. The situation is even more complicated in the neonates on mechanical ventilation. No standard values were estimated for these children and the roles of those indices in assessing fluid responsiveness in children on mechanical ventilation remain uncertain. We did not find any statistical difference between the

groups. Some of our patients were intubated during the initial echocardiography (Table 1).

Mourani et al studied the clinical utility of echocardiography for the diagnosis and management of pulmonary hypertension. They compared the echocardiography data in infants with Group 1 with catheterisation data. They concluded that echocardiography correctly diagnosed the presence or the absence of pulmonary hypertension in children (79%) under the age of 2 but the severity of pulmonary hypertension was measured accurately only in 47%.¹⁸ Similar to Mourani et al, Slaughter et al¹⁹ explained that echocardiogram was limited in their ability to differentiate the severity of pulmonary hypertension. Despite this limitation, they said that it was still considered the best method to screen bronchopulmonary dysplasia patients for pulmonary hypertension at the population level. These data show that echocardiography only is not enough to follow bronchopulmonary dysplasia patients for pulmonary hypertension. Serum biomarkers are always popular in the diagnosis and the follow-up of pulmonary hypertensive patients. The oldest and most popular one was N-terminal proBrain natriuretic peptide which was shown to be correlated with systolic pulmonary artery pressure in paediatric population.^{20,21} Kim et al reported that N-terminal-proBrain natriuretic peptide must be measured in bronchopulmonary dysplasia patients with greater than moderate degree to prevent pulmonary hypertension and to ensure early treatment if pulmonary hypertension is present.²² The other biomarker that we used in our study was kallistatin. In our previous study,²³ we have shown that kallistatin levels were significantly lower ($p < 0.05$) in pulmonary hypertensive patients especially in the ones with Eisenmenger syndrome. In that study, we detected negative correlation between systolic pulmonary artery pressure and serum kallistatin levels. In this study, significant difference was detected between Group 1 and Group 2 (control). There is no significant difference between Group 1 and Group 3. Actually, the decrease in kallistatin levels can be explained by pulmonary hypertension. But its levels were also low in Group 3. This can be explained as the underlying lung disease lead to the decrease in kallistatin levels. As an assumption, probably the patients with bronchopulmonary dysplasia have pulmonary hypertension which is not overt and not detected by echocardiography; however, serum kallistatin levels were lowered. In addition to these, we have looked serum biomarkers at the beginning of the study and not repeated. Therefore, we do not know how the levels of kallistatin change over time in these children. Serum homocysteine, cystatin C, and gelsolin levels were also measured in our study. High homocysteine levels have been associated with endothelial dysfunction in both clinical and preclinical studies.²³ The study of Sun et al²⁵ showed that homocysteine had good sensitivity and specificity to predict pulmonary hypertension associated with CHDs, we could not find any statistical significance in homocysteine values between groups. Cystatin C is an endogenous marker actually used for renal function; however, it is used for right ventricular systolic pressure pressures, function, and morphology. NT-probrain natriuretic peptide levels were found to be positively correlated with right ventricular systolic pressure systolic pressure, right ventricular systolic pressure strain, and right ventricular systolic pressure strain rate.²⁵ However, there was no study telling the role of cystatin C in Group 1 patients. In our study, there was no statistical difference in cystatin C levels between the groups.

The reason that we did not find any significance in serum cystatin C and probrain natriuretic peptide, homocystein levels

were the size of our samples. Larger cohorts need to be studied to make good validation for these biomarkers.

Conclusion

Echocardiography is a good non-invasive method to screen the preterm infants with bronchopulmonary dysplasia for pulmonary hypertension. In this study, we have accomplished to evaluate the echocardiographic measurements with serum biomarkers. Previous studies have shown that using only echocardiographic data can be restrictive in the diagnosis and follow-up of pulmonary hypertension. Therefore, we thought that serum biomarkers can be an important team player besides echocardiography. The only restriction is the size of our samples which are small and hard to make a validation.

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