Pulmonary hypertension grading in the neonate: Pediatric autopsy series compared with etiology of lung disease

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Backgrounds and aim: Deciding the main diseases and the cause of death in pediatric autopsies is very important and sometimes difficult. Cardiopulmonary system is very carefully observed in autopsies. Besides exploration of the lung parenchyma, vasculature of the lungs must be observed as well. In this study we searched for the relation of pulmonary hypertension (PHT) grading score and the main disease of the lung in pediatric autopsy cases.

Materials and methods: Sections from both left and right lobes of lungs from 188 pediatric autopsy cases were searched for pulmonary hypertension and graded according to “Health and Edwards Grading Classification” on both hematoxylin and eosin; and Verhoeiff’s elastic stains. The cases were grouped as pulmonary infection (PI), meconium aspiration and/or hyaline membranous disease (MAHMD), congenital heart disease (CHD), diaphragmatic hernia (DH) and/or pulmonary hypoplasia (PH), and control group. Differences in PHT grades were searched by Kruskal Wallis test, and compared with control group for each group by using a Student’s unpaired T test for groups with over 30 cases, and Mann Whitney U test was used if any group of the bivariate testing is under 30 cases. P< 0.05 was considered significant.

Results: A hundred cases (53.2%) were male, 88 cases (46.8%) were female. In 57 cases (30.3%) no vascular abnormality was observed. In 76 cases (40.4%) grade I lesions, while in 45 cases (23.9%) grade II lesions and in 10 cases (5.3%) grade III lesions existed. PHT grading scores were higher in DHPH group (p=0.0001), CHD group (p=0.0001), MAHMD group (p= 0.002) and PI group (p=0.004) when compared with control group. No clinical knowledge was known before death in any of the cases.

Conclusion: We conclude that the end stage changes during death do not cause PHT, although induces cardiopulmonary stress and hemorrhage. This study shows that PHT is induced perinatally by stress and hypoxia especially in cardiopulmonary disease; even PI or MAHMD may cause a start in PHT. But especially PHT grade is higher in DHPH and CHD group.

Key words: Pulmonary hypertension, pediatric autopsies, histological grading

Introduction

Pulmonary hypertension (PHT) in the newborn period or later in life may be caused by a wide variety of conditions, such as congenital heart disease (CHD), meconium aspiration (MA), bronco-pulmonary dysplasia and pulmonary hypoplasia (PH).¹,²

PHT may be primary or secondary to another disease. Primary PHT is a rare genetic disease. The most common secondary forms of PHT in children are due to persistence of neonatal anatomy (neonatal PHT), to heart diseases with left-right shunt to diseases of the pulmonary parenchyma (interstitial viral infection, mucoviscidosis), and complications of heart surgery. PHT is associated with a variety of structural and functional changes of the pulmonary arteries. These structural changes include medial hypertrophy of the arterial wall and narrowing of the
vascular lumina by intimal thickening, resulting in an increase in pulmonary vascular resistance. The histologic grading classifications of Heath and Edwards (1958) and Wagenvoort (1981) and World Health Organization (WHO) (1998) are widely used to evaluate the changes of PHT.3–6

Pulmonary plexogenic arteriopathy (PPA) is a specific form of pulmonary vascular disease. In contrast to other arteriopathies such as those associated with chronic hypoxia or pulmonary venous congestion, PPA may ultimately cause characteristic vascular alterations, including concentric-laminar intimal fibrosis, dilatation of lumens, fibrinoid necrosis of small arteries, and plexiform lesions. Dilatation and plexiform lesions, which are regarded as indicative of irreversibility of the vascular disease, are characterized by local abnormal vasodilatation and endothelial cell proliferation. Plexiform arteriopathy and laminar intimal fibrosis are rare in infants, when present they are usually focal.1,2,7

Lung hypoplasia and a pattern of alveolar simplification occur in several neonatal lung diseases including bronchopulmonary dysplasia, congenital diaphragmatic hernia, and Down’s syndrome. These diseases are also associated with an increased risk for development of PHT.3

Although grading of PHT is not correlated with clinical findings in cases other than congenital heart disease, PHT is sometimes observed in other disease. In the present study we searched for the relation of PHT grading score and the main disease in pediatric autopsy cases to search for whether end stage death cardiopulmonary stress may cause PHT vascular changes or existence of PTH findings in the lung predicts a perinatal stress.

Materials and methods

In this study lung sections of 188 pediatric autopsy cases were studied for pulmonary vascularity. Gestational periods of all cases were greater than 35 weeks. The sections were obtained from archival paraffin blocks of pediatric pathologic autopsy cases performed between 1989 and 2003.

Small pieces of left and right lungs had been placed in 10% buffered formalin and paraffin-embedded. Paraffin sections 5µm thick were serially mounted onto slides. Hematoxylin and eosin staining was performed. At least three sections per left and right lungs for each case were assessed for histologic changes. Verhoeff’s elastic stain8 was applied for one demonstrative slide of each case. For these histochemical staining, slides were dewaxed in 100% xylene. Sections were rehydrated by immersion in 100%, 95%, 70% ethanol, and then water. The sections were stained in Verhoeff’s iron hematoxylin for 20 minutes. No differentiation was needed because nuclei and fibers were black and the background was weakly stained. After washing in water, 95% ethanol was applied. Counterstaining was done in van Gieson’s stain for 3 minutes. After rapid dehydration, sections were mounted and observed. The reasons of death, age, sex, main disease, and pulmonary pathology were gathered from archive records.

The cases were grouped as pulmonary infection (PI), meconium aspiration and/or hyaline membranous disease (MAHMD), congenital heart disease (CHD), diaphragmatic hernia and/or pulmonary hypoplasia (DHPH), and control group (with disease other than cardiopulmonary system such as urinary, gastrointestinal or central nervous system disease). Heath and Edwards grading was used (Table 1). Pulmonary arteries were evaluated by two investigators blinded for the clinical data. Confirming the localization of intima, media, elastic lamina were available by Verhoeff’s elastic stain. Differences in PHT grades were searched by Kruskal Wallis test. After that comparison with control group for each group by using a Student’s unpaired t test for groups with over 30 cases was done. Mann Whitney U test was used if any group is under 30 cases. p< 0.05 was considered significant.

Table 1. The Heath-Edwards grades of pulmonary hypertension

<table>
<thead>
<tr>
<th>Histology</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
<th>Grade 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscular extension into arterioles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Medial hypertrophy of muscular arteries</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Medial hypertrophy with intimal cellular proliferation</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Progressive intimal fibrosis and occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plexiform lesions and dilation of arteries</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Chronic dilatation lesions with veinlike arteries</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Arterial necrosis and arteritis</td>
<td></td>
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</tbody>
</table>

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Results

In this series 100 cases (53.2%) were male, while 88 cases (46.8%) were female. The mean age of the cases was 26.56±112.324 days. The main diseases and relationship with PHT are given in Table 2. In 57 cases (30.3%) no vascular abnormality or only adventitial changes were observed. In 76 cases (40.4%) grade I lesions (Figure 1), while in 45 cases (23.9%) grade II lesions (Figure 2) and in 10 cases (5.3%) grade III lesions (Figure 3) were observed. As seen many of the cases were grade I or II. Grade III lesions were very rare and focal. There was no difference in the grades of PHT in right and left lungs for each case.

In independent T test PHT grading scores were higher in DHPH group (p=0.0001), MAHMD group (p= 0.002) and in PI group (p=0.004) and in Mann Whitney U test higher in CHD group (p=0.0001), when compared with control group.

Table 2. Main disease and pulmonary hypertension.

<table>
<thead>
<tr>
<th>Main disease</th>
<th>n</th>
<th>%</th>
<th>Mean grade</th>
<th>Mean Rank (Kruskal-Wallis test)</th>
<th>P (compared with control group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>48</td>
<td>25.5</td>
<td>0.17</td>
<td>40.08</td>
<td>0.004</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td>47</td>
<td>25.0</td>
<td>1.06</td>
<td>95.35</td>
<td>0.002</td>
</tr>
<tr>
<td>Meconium aspiration and/or hyaline membranous disease</td>
<td>40</td>
<td>21.3</td>
<td>1.23</td>
<td>106.84</td>
<td>0.002</td>
</tr>
<tr>
<td>Congenital Heart Disease</td>
<td>23</td>
<td>12.2</td>
<td>1.44</td>
<td>132.36</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diaphragmatic hernia and/or pulmonary hypoplasia</td>
<td>30</td>
<td>16.0</td>
<td>1.70</td>
<td>134.30</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Figure 1. Grade I pulmonary hypertension: A pulmonary artery with medial hypertrophy and adventitial changes (Verhoeff’s Elastic stain, x400)

Figure 2. Grade II pulmonary hypertension: A pulmonary arteriopathy with medial hypertrophy and intimal thickening (Verhoeff’s Elastic stain, x400)

Figure 3. Grade III pulmonary hypertension: A small pulmonary artery in the area of a bronchiole with almost complete occlusion of the vascular lumen (HE, x400)
Discussion

Normally, there is a postnatal fall in pulmonary vascular resistance. If a failure has become in this process, persistent pulmonary hypertension occurs. In the neonate with PHT, elevated pulmonary vascular resistance rather than increased pulmonary blood flow is the main pathophysiologic feature. Depending on the cause and duration of PHT, muscular thickness in the vessel wall with or without endothelial changes may be determined. Medial hyperplasia on the vessel wall is the initial change of PHT and develops with extension of smooth muscle in the medial layer of vessel wall. In many peripheral arteries, the lumina are narrowed and the thickness of the walls is about twice normal. It is thought that these arterial changes begin in utero, as a consequence of increased sensitivity to hypoxia or stress, alterations of pulmonary flow characteristics or primary failure of mechanisms governing arterial muscularisation and tone.1–3

Although the mechanisms that coordinate lung vascular growth with alveolarization are uncertain, the imbalance of some substances and regulator mechanisms are contributed to the pathophysiological changes in perinatal pulmonary hypertension by favoring increased vasoconstriction and smooth muscle proliferation. Such as vasoactive substances, including prostaglandins may play roles in the development of the muscularisation of the intraacinar vessels and abnormal platelet aggregation. Similarly signal from air way epithelial cells are thought to play a major role. Experimental models of PHT showed that hypertension down regulated vascular endothelial growth factor (VEGF) expression in the developing lung and impaired VEGF signaling might contribute to the pathogenesis of persistent PHT of the newborn.9–11

PHT may rarely cause sudden death as an initial manifestation of the disease. There are a few pediatric cases reported with sudden death. Chronic intrauterine PHT is thought to cause elevation of intrauterine pulmonary artery pressure, right ventricular hypertrophy and hypertensive lung structural changes. In the present study sudden death was not due to PHT in any of the cases. But it is advised that, in the diagnosis of primary PHT, particularly in forensic cases a careful evaluation of multiple sections of lung must be performed and the vasculature should be examined being aware of the possibility of PHT. It is known that lung biopsy and PHT grading could play an important role in determining the reversibility of pulmonary vascular obstruction, particularly in patients older than 2 years. Recent studies suggest that PHT will be reversible when arterial lesions are limited to medial hypertrophy.12

PPA is a peculiar form of pulmonary vascular disease associated with chronic hypoxia or pulmonary venous congestion. Therefore PPA may develop in patients with congenital heart disease, where increased pulmonary blood flow appears to be an obligatory factor and elevated pulmonary artery pressure seems to accelerate disease progression. Recently, Heath and Edwards grading system has been criticized because it does not take into consideration the clinical features of child and the presence of plexogenic arteriopathy. But also this features now considered important prognostic marker. Plexogenic arteriopathy and laminar intimal fibrosis are rarely seen in the pediatric cases. Similarly in our series, PPA was not determined in any of the cases.7,13

Lung hypoplasia, PHT, biochemical and structural immaturity of the lungs are associated with CDH. In CDH, there is failure of the normal arterial remodeling processes occurring in the perinatal period. Pulmonary vascular morphology in CDH does not differ between the groups with lung hypoplasia or persistent pulmonary hypertension as primary cause of death. This study shows that PHT is induced perinatally by stress and hypoxia especially in cardiopulmonary disease, even PI or MAHMD may cause a start in PHT. But especially PHT grade is higher in DHPH and CHD group. In autopsy or biopsy sections of lung, the vascularisation must be carefully observed as well as the parenchyma. Low grade changes on the vessel walls do not always indicate cardiopulmonary malformation. A prenatal long duration hypoxia or stress might also be observed with changes such as medial hypertrophy. It would be useful to study on growth factors and hypoxia induced factors on the basis of genomics or proteomics in such neonatal cases.14–17 Analyzes using morphometry in lung biopsy specimens may be used especially in
evaluating pulmonary vascular remodeling process after reperative surgery.\(^{18}\)

**References**


