Cardiology in the Young

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Leukotriene B4 levels in CHD-associated paediatric pulmonary hypertension

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Original Article

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Abstract

Background: The aim of this study is to evaluate the role of leukotriene B4, an inflammatory mediator, in the development of pulmonary hypertension in paediatric patients with CHD with left-right shunt. *Methods:* The study included forty patients with CHD with left-right shunts. Based on haemodynamic data obtained from cardiac diagnostic catheterisation, 25 patients who met the criteria for pulmonary arterial hypertension were included in the patient group. The control group comprised 15 patients who did not meet the criteria. The standard cardiac haemodynamic study was conducted. Leukotriene B4 levels were assessed in blood samples taken from both pulmonary arteries and peripheral veins. *Results:* The median age of patients with pulmonary arterial hypertension was 10 months (range: 3–168), while the median age of the control group was 50 months (range: 3–194). In the pulmonary hypertension group, the median pulmonary artery systolic/diastolic/mean pressures were 38/18/24 mmHg, compared to 26/10/18 mmHg in the control group. Leukotriene B4 levels in pulmonary artery blood samples were significantly higher in the pulmonary arterial hypertension group compared to the controls ($p < 0.05$). Peripheral leukotriene B4 levels were also elevated in the pulmonary arterial hypertension group in comparison to the control group, though the difference was not statistically significant. *Conclusion:* The discovery of elevated leukotriene B4 levels in pulmonary artery samples from paediatric patients with pulmonary arterial hypertension secondary to CHD with left-to-right shunt suggests that local inflammation may have a pathological role in the development of pulmonary arterial hypertension.

Pulmonary arterial hypertension is a progressive and life-threatening disease characterised by increased pulmonary vascular resistance and pulmonary arterial pressure resulting from vasoconstriction and abnormal vascular remodelling. Vasoconstriction, genetic, metabolic dysregulation, and inflammation are all involved in the pathogenesis of the disease.[1–6] Perivascular inflammation is commonly observed in all forms of pulmonary arterial hypertension.[7–10] This inflammation is marked by the immune cells, including T cells, B cells, mast cells, plasma cells, and macrophages, as well as inflammatory molecules such as cytokines, chemokines, eicosanoids, growth factors, and reactive oxygen.[11,12]

Leukotrienes are the primary eicosanoid products of leukocytes and serve as vital mediators of inflammation. They are synthesised from arachidonic acid through the 5-lipoxygenase pathway.[13] The 5-lipoxygenase enzyme, in conjunction with 5-lipoxygenase activating protein, converts arachidonic acid to leukotriene A4. Leukotriene A4 is rapidly hydrolysed by leukotriene A4 hydrolase to leukotriene B4. Leukotriene B4 is a potent chemoattractant, particularly for neutrophils and macrophages. Due to their pathological effects, such as increased matrix protein production, enhanced smooth muscle contractility and cell proliferation, and increased cell survival, leukotrienes have been shown to play pivotal roles in various lung diseases, including asthma, pulmonary fibrosis, and chronic pulmonary hypertension.[14–16]

Animal experiments involving adult rat models have been published, suggesting that leukotrienes may play a critical role in chronic pulmonary hypertension.[17–19] There are also studies in human subjects, particularly in the adult age group, investigating the effect of leukotriene B4,[15,18,20] especially in pulmonary hypertension secondary to connective tissue diseases. Additionally, research has been conducted in the paediatric age group, focusing on newborns with bronchopulmonary dysplasia.[21,22] In the paediatric age group, pulmonary arterial hypertension often develops due to left-right shunt cardiac defects. In this study, we aimed to evaluate the contribution of leukotriene B4 to pulmonary hypertension in paediatric patients with pulmonary arterial hypertension secondary to CHDs with left-right shunts.

Method

In this prospective, single-centre study, we enrolled patients aged 0–21 years with a diagnosis of CHD with pre-tricuspid (atrial septal defect), post-tricuspid (ventricular septal defect, patent ductus arteriosus), or combined (atrioventricular septal defect) left-right shunts. These patients were admitted to the Department of Pediatric Cardiology, Dr Behçet Uz Children's Hospital, Izmir, between November 2020 and November 2021 and were scheduled to undergo diagnostic or interventional catheter angiography. As per our centre, preoperative catheter angiography is routinely conducted for all patients aged over 6 months scheduled for surgical closure of ventricular septal defects to assess the presence of pulmonary hypertension.

Diagnostic catheter angiography is more frequently employed due to the suboptimal image quality of MR and CT imaging in our centre, particularly in small patients where motion artefacts are prevalent. We used the 6th World Symposium on Pulmonary Hypertension (NICE 2018) haemodynamic diagnostic criteria to define pulmonary arterial hypertension, which includes criteria such as mean pulmonary artery pressure > 20 mmHg, pulmonary artery wedge pressure ≤ 15 mmHg, and pulmonary vascular resistance index ≥ 3WUxm2. According to these criteria, we included 25 patients in the patient group who met the criteria for pulmonary arterial hypertension, while 15 patients who did not meet these criteria formed the control group. Patients with idiopathic pulmonary hypertension, persistent pulmonary hypertension, and systemic connective tissue disease were excluded from the study. All patients were informed about the study before catheterisation, and only those who provided informed consent were included. During cardiac catheterisation, blood samples were collected from both the pulmonary artery and a simultaneous peripheral vein. These blood samples were centrifuged and stored at −70°C. The concentration of leukotriene B4 was determined using the leukotriene B4 enzyme immunoassay kit provided by Cayman Chemical, following the manufacturer's protocol. Patients from whom appropriate blood samples could not be obtained or whose blood samples could not be analysed (due to storage conditions, etc.) were excluded from the study. The study received ethics committee approval from the Local Ethics Committee for Clinical Research at Dr Behçet Uz Children's Diseases and Surgery Training and Research Hospital.

Statistical analysis

Statistical analyses within the scope of this study were carried out using the SPSS 26.0 package program (SPSS Inc., Chicago, IL, USA). Before the analyses, the normality of the distribution in the measurement data was assessed through the Kolmogorov-Smirnov and Shapiro-Wilk tests. The results of the normality tests indicated that none of the measurements followed a normal distribution. Under these circumstances, non-parametric tests were deemed appropriate. Inter-group comparisons were performed using the Mann-Whitney U test, and the correlation between measurement values was assessed using Spearman correlation analysis. Binary logistic regression analysis was employed in the evaluation of leukotriene B4 levels in the pulmonary artery and peripheral vein in relation to the pulmonary arterial hypertension and control group. The statistical significance level for this study was set at $p < 0.05$.

Results

A total of 40 patients were included in the study, comprising 25 patients with pulmonary hypertension (15 girls and 10 boys) and 15 control subjects (6 girls and 9 boys). The median age of the pulmonary hypertension group was 10 months (range: 3–168), while the median age of the control group was 50 months (range: 3–194). The median body weight of the pulmonary arterial hypertension group was 8.9 kg (range: 5–50), and the median body weight of the control group was 14 kg (range: 5–53). The majority of patients in the pulmonary hypertension group were diagnosed with patent ductus arteriosus. These patients were primarily in the younger age group and presented with moderate to large patent ductus arteriosus. In the pulmonary hypertension group, only two patients were receiving anti-pulmonary arterial hypertension therapy, with one patient on sildenafil and the other on bosentan. The patients' demographic, clinical, and haemodynamic data are summarised in Table 1. Detailed information regarding pulmonary arterial pressures and leukotriene B4 levels of patients in the pulmonary hypertension group has been provided as supplemental data in Table 2.

Table 1. Demographic, clinical, and haemodynamic data of patients

	PAH (n:25)	Control (n:15)
Age (months, range)	10 (3–168)	50 (3–194)
Gender (F/M)	15/10	6/9
Weight (kg, median, range)	8.9 (5–50)	14 (5–53)
Diagnosis		
ASD	1	4
VSD	7	2
AVSD	1	–
PDA	16	9
Pulmonary artery systolic pressure (mmHg, median,range)	38 (25–92)	26 (19–32)
Pulmonary artery diastolic pressure (mmHg, median, range)	18 (10–44)	10 (5–16)
Pulmonary artery median pressure (mmHg, median, range)	24 (21–66)	18 (11–20)

ASD = atrial septal defect; VSD = ventricular septal defect; AVSD = atrioventicular septal defect; PAH = pulmonary arterial hypertension; PDA = patent ductus arteriosus.

In the pulmonary arterial hypertension group, the median pulmonary artery systolic pressure was 38 mmHg, the median diastolic pressure was 18 mmHg, and the median mean pressure was 24 mmHg. In the control group, the median pulmonary artery systolic pressure was 26 mmHg, the median diastolic pressure was 10 mmHg, and the median mean pressure was 18 mmHg. Pulmonary artery leukotriene B4 levels were significantly higher in the pulmonary arterial hypertension group compared to the control group (respectively, 45 (range: 4–1300) versus 24 (range: 15–65) pg/ml $p < 0.05$). Peripheral leukotriene B4 levels were also higher in the pulmonary arterial hypertension group compared to the control group, though the difference was not statistically significant. (respectively, 33 (range: 6–351) versus 30.1 (range: 24–59) pg/ml, $p > 0.05$).

Vasoreactivity testing was performed in nine patients in the pulmonary hypertension group, with positive results obtained in six patients and negative results in three patients. In patients with negative vasoreactivity test, the mean leukotriene B4 levels in the pulmonary artery were 23.2 ± 19.6 pg/ml and 36.7 ± 39.2 pg/ml in the peripheral vein. In contrast, in the positive vasoreactivity test

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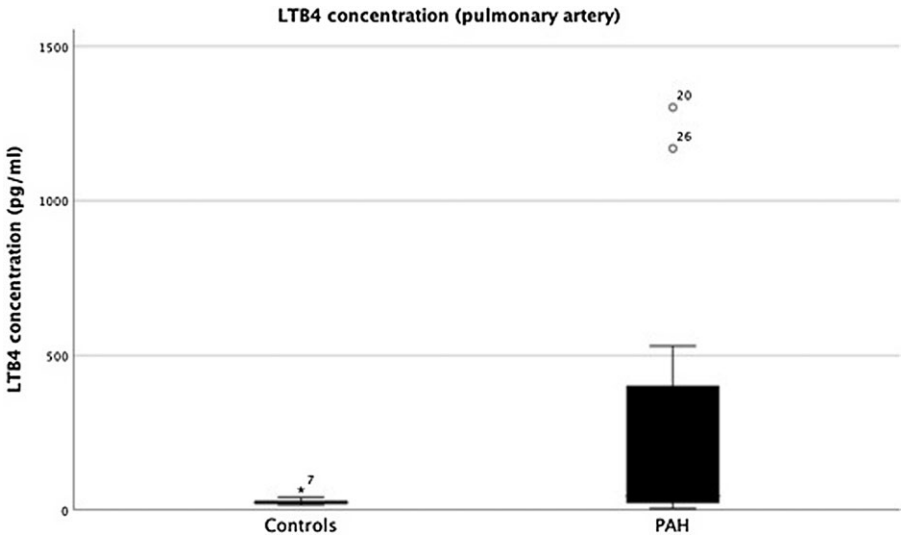

Figure 1. LTB4 levels in pulmonary arter.

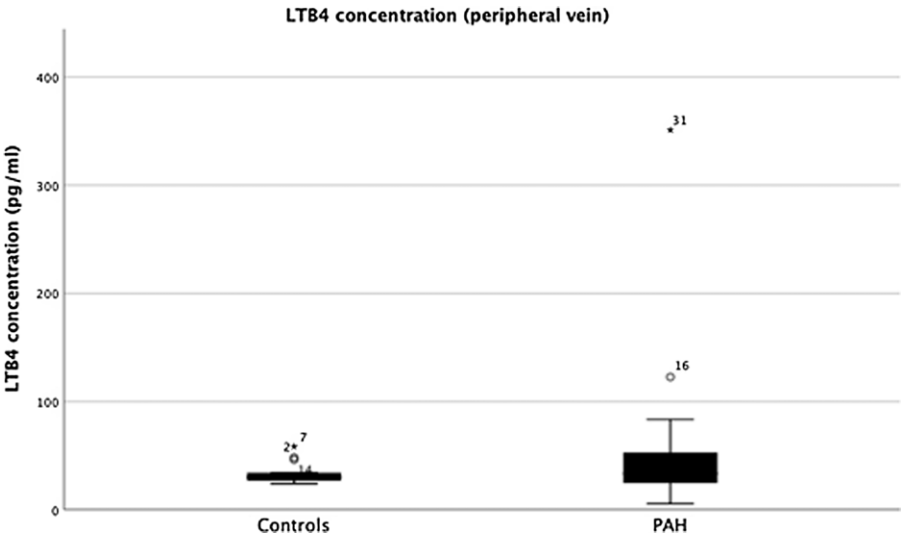

Figure 2. Leukotriene B4 levels in peripheral vein.

group, the mean leukotriene B4 levels in the pulmonary artery were 27.5 ± 20 pg/ml and in the peripheral vein 28.3 ± 16 pg/ml. Owing to the limited number of patients, a statistical comparison between these two groups could not be conducted.

Regression analyses did not reveal any statistically significant association between pulmonary arterial hypertension severity or length and leukotriene B4 serum concentration ($r:-0.05$, $p > 0.05$ and r: 0.55, $p > 0.05$, respectively). Leukotriene B4 level analyses are illustrated in Figure 1 and Figure 2.

Discussion

In recent years, there has been a growing interest in evaluating pathobiological concepts such as cell damage, repair mechanisms, and interactions within complex multicellular systems, alongside mechanical factors like pressure, shear stress, blood flow, and right ventricle wall tension in the pathophysiology of pulmonary arterial hypertension.[23] Irregularities in regional blood flow within the lung as a result of hyperdynamic circulation, left-right shunt, and pulmonary vasoconstriction cause endothelial damage and induce apoptosis. Pulmonary hypertension develops due to proliferation of endothelial cells, resistance to apoptosis, and pulmonary vascular remodelling during inappropriate wound healing.[24] It is now widely acknowledged that inflammation significantly influences susceptibility and the progression of vascular remodelling in pulmonary arterial hypertension.[25] Within this context, there has been an increased focus on studies investigating the impact of leukotriene B4, an inflammatory marker, on the development of pulmonary arterial hypertension.[15,17–20]

In the paediatric age group, pulmonary arterial hypertension often develops secondary to CHDs with left-right shunts. Numerous studies have demonstrated the impact of inflammation in the pathogenesis of pulmonary arterial hypertension associated with CHD. These studies have consistently shown significantly elevated levels of inflammatory markers such as IL-1B, IL-6, TNF-a, and TGF-B in patients with CHD-associated pulmonary arterial hypertension compared to control groups.[26,27] However, no literature appears to have explored the impact of leukotriene B4 on the pathogenesis of pulmonary arterial hypertension in both paediatric pulmonary arterial hypertension and CHD-associated pulmonary arterial hypertension groups. In our investigation, leukotriene B4 levels in the pulmonary arteries were notably higher in the pulmonary arterial hypertension group compared to the

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control group. Pulmonary arterial hypertension groups' leukotriene B4 levels in peripheral blood were also elevated, although the difference was not significant. Both in human and animal experiments, it has been observed that macrophages secreting leukotriene A4 hydrolase, the precursor of leukotriene B4, are concentrated around diseased arterioles.[18,28] This observation suggests the involvement of leukotriene B4 in local lung inflammation. Given the brief half-life of leukotrienes,[29] we hypothesise that the peripheral dissemination of leukotriene B4-induced pulmonary inflammation may be constrained, consistent with our study's findings.

No study providing reference values for leukotriene B4 levels in healthy children was identified in the literature. In studies where the effect or level of leukotriene B4 was measured, results were consistently assessed compared to control groups. These studies reported leukotriene B4 levels in the control group ranging from 0 to 25 pg/ml.[30–32] In our study, we included patients with CHD without pulmonary arterial hypertension as our control group, and the leukotriene B4 levels in our control group were in line with the control groups in previous studies.

Regression analyses did not reveal a meaningful relationship between the severity or duration of pulmonary arterial hypertension and leukotriene B4 levels. This might be attributed to the limited number of patients in our study.

Initially, Voelkel et al. demonstrated an increase in 5-lipoxygenase expression in both alveolar macrophages and vascular endothelial cells in rats exposed to chronic hypoxia.[19] Subsequently, Wright et al. reported elevated 5-lipoxygenase levels in the lungs of patients with pulmonary arterial hypertension compared to healthy controls.[20] In another study by Tian et al., the levels of leukotriene B4 in bronchoalveolar lavage fluids of rats in the pulmonary hypertension group were significantly elevated compared to healthy rat controls. They also illustrated that leukotriene B4 induced endothelial cell apoptosis, promoted smooth muscle cell proliferation and hypertrophy, and caused proliferation in pulmonary artery adventitial fibroblasts. Their study revealed that leukotriene B4 levels were significantly higher in 19 pulmonary arterial hypertension patients in whom leukotriene B4 levels were measured, particularly in patients with pulmonary arterial hypertension secondary to connective tissue diseases. They added that leukotriene B4 levels were within the normal range in six out of eight idiopathic pulmonary arterial hypertension patients.[18,28]

Studies investigating the effect of leukotriene B4 on pulmonary hypertension are frequently conducted in animal models, examining the molecule's effects at the cellular level. These investigations often involve the assessment of bronchoalveolar lavage fluids or lung tissue samples from rats. However, in human studies, particularly in paediatric patients, obtaining such samples for research can be highly invasive. Our study assessed leukotriene B4 levels in blood samples taken from both peripheral veins and the pulmonary artery, where we believed inflammation might be more pronounced. As a result of our study, leukotriene B4 level was significantly higher in the patient group compared to the controls, especially in pulmonary artery blood samples. This result supports the notion that lung and right ventricular inflammation may contribute to the pathobiology of pulmonary arterial hypertension.

Studies have reported elevated levels of inflammatory markers, such as IL-10, IL-1B, TNF-a, and fractalkine, in CHD-pulmonary arterial hypertension patients.[33,34] Nevertheless, these studies have failed to comprehensively explain the observed heightened inflammation in this patient group. The underlying cause of increased inflammation in pulmonary arterial hypertension patients with CHD, and whether inflammation is a cause or an effect in the pathogenesis of pulmonary arterial hypertension, remains unclear. Stress-induced factors, including shear stress, oxidative stress, or tension on the right ventricle, may activate endothelial cells, releasing pro-inflammatory cytokines and growth factors.[20] Given this context, randomised controlled trials involving leukotriene B4 blockade are essential to differentiate whether the elevated leukotriene B4 levels identified in our study are merely an epiphenomenon or a pivotal mechanism in the pathogenesis of pulmonary arterial hypertension.

Study limitations

Our study is a single-centre study conducted with a limited number of patients. This study evaluated only leukotriene B4 levels in patients with CHD-associated pulmonary arterial hypertension. However, we did not elucidate the role of the leukotriene B4 molecule on the pathogenesis, its cellular-level mechanisms of action, or its relationship with the clinical outcomes and prognosis of patients with CHD-associated pulmonary arterial hypertension. In our study, two (8%) patients were receiving anti-pulmonary arterial hypertension therapy. We could not entirely eliminate the possibility of anti-pulmonary arterial hypertension therapies influencing leukotriene B4 molecule levels, serving as a potential confounding factor.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1047951124000362.

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Competing interests. None.

Ethical standards. Approval for the study was obtained from the Clinical Research Ethics Committee of the University of Health Sciences, İzmir Dr Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital.

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