

TNF- α as a predictive factor of pulmonary hypertension in children with Down syndrome with and without congenital heart disease

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Abstract

Background Down syndrome (DS) is a chromosomal disorder due to trisomy 21 that may involve congenital heart disease (CHD). Pulmonary hypertension (PH) may be present in DS with and without CHD. TNF- α is a cytokine involved in the pathogenesis of inflammation in PH.

Objective To determine the association between TNF- α and the risk of PH in children with DS with and without congenital heart disease.

Methods This observational study was conducted in DS children aged two months to five years who visited the outpatient clinic of a regional referral hospital in Indonesia. Subjects underwent echocardiography and were classified into four groups (CHD-PH, CHD-no PH, no CHD-PH, no CHD-no PH). Serum TNF- α was measured in all subjects. We used the ANOVA test to compare mean TNF- α between the groups and to determine the optimal TNF- α cut-off point. We compared the risk of PH in subjects with TNF- α above and below the cut-off point.

Results We included 36 DS children in this study. Mean TNF- α in the CHD-PH, CHD-no PH, no CHD-PH, and no CHD-no PH groups was 2,564.44 (SD 177.00) pg/mL, 2,112.89 (SD 382.00) pg/mL, 2,211.56 (SD 330.70) pg/mL, and 1,118.89 (SD 1056.65) pg/mL, respectively ($P < 0.001$). The optimal TNF- α cut-off point was 2,318 pg/mL. DS children with TNF- $\alpha \geq 2,318$ pg/mL had a higher risk of CHD (RR=2.6; 95%CI 1.17 to 5.78; $P=0.008$) and PH (RR=3.5; 95%CI 1.43 to 8.60; $P=0.001$).

Conclusions DS children with CHD accompanied by PH have significantly higher TNF- α levels than those without PH and those without CHD. In children with DS, an elevated TNF- α level ($\geq 2,318$ pg/mL) is associated with a higher risk of CHD and PH. [Paediatr Indones. 2022;62:61-5; DOI: 10.14238/pi62.1.2022.61-5].

Keywords: Down syndrome; pulmonary hypertension; TNF- α

Down syndrome (DS) is a genetic syndrome caused by trisomy of chromosome 21. Its prevalence ranges from 1:700 to 1:1,000 live births.¹ Individuals with DS are prone to oxidative damage as they lack adequate cell repair ability. Down syndrome, along with upper respiratory tract obstruction, congenital heart disease (CHD), and obstructive sleep apnea (OSA), can trigger hypoxic conditions.^{2,3} Hypoxia induces the p38 MAPK pathway, hypoxia-inducible factor-1 α (HIF-1 α), and reactive oxygen species (ROS), which triggers the increase of TNF- α levels.^{4,5} Chronic infections, which often accompany DS, can stimulate TNF- α production.² Hypoxia decreases the expression of BMPR2 signals. Mutations of the BMPR2 gene have been reported in trisomy 21 patients with pulmonary hypertension (PH) due to CHD.⁶ Other studies have also found mutations or deficient expression of BMPR2 in cases of CHD without PH.⁷⁻⁹

Pulmonary hypertension (PH) is characterized by an increase in blood pressure within the pulmonary arteries of ≥ 25 mmHg at rest or ≥ 30 mmHg during

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exercise. It is commonly associated with heart, pulmonary, and systemic diseases,^{10,11} and is caused by endothelial dysfunction, with BMPR2 mutations as the most common genetic cause.^{7,12} The lack of BMPR2 gene expression can stimulate TNF- α activation through microRNA (miR) and destruction of metalloprotease-mediated receptors, resulting in cellular metabolic damage, mitochondrial dysfunction, glucose oxidation and glycolysis, potentiation of pyruvate kinase splicing alternatives, and decreased mitochondrial oxidative phosphorylation.⁸

TNF- α is a mediator which increases during inflammation.¹³ Previous studies have shown that TNF- α decreases BMPR2 expression in patients with PH, is associated with reduced expression of BMPR2 and cleavage of BMPR2 in vascular endothelium, and play a role in the improper proliferation of BMPR2.^{5,6,14} In this study, we aimed to assess the value of TNF- α as a predictive factor of PH in children with DS.

Methods

This observational study was performed in 36 children with DS aged between two months and five years who visited the outpatient clinic of Moewardi Hospital, Surakarta, Indonesia from March to April 2020. All DS pediatric patients with or without CHD were included in our study and classified into four groups: CHD-no PH, CHD-PH, no CHD-PH, and no CHD-no PH. Parents provided written informed consent. Exclusion criteria were post-closure of heart defect either by open heart procedure or occluder insertion, clinical signs of right heart failure characterized by anasarca, hepatomegaly, increased jugular venous pressure, permagna ascites, Eisenmenger's syndrome characterized by a right-to-left shunt or central cyanosis, and critical congenital heart disease including truncus arteriosus, left ventricular hypoplasia, and transposition of the great arteries. Subjects underwent blood laboratory examinations and echocardiography. Blood serum specimens were centrifuged at 3000 rpm and plasma stored in 1 mL microtube at -70°C. TNF- α levels were measured using an ELISA kit (*Wuhan Elab Science*, China) at the laboratory of the Universitas Sebelas Maret Medical School. We used the analysis of variance (ANOVA) test was used to obtain the optimal TNF- α cut-off point and the chi-square test to compare

the risk of CHD and PH in subjects with TNF- α levels above and below the cut-off point. Data were analyzed using SPSS 22 (IBM, Armonk, New York) and a p value of <0.05 was considered to be statistically significant.

Results

Thirty-six patients met the inclusion criteria during the study period. Most subjects were male (21/36; 58%). With regards to CHD, 18/36 subjects (50%) had no CHD, 9/36 (25%) had atrial septal defect (ASD), 4/36 (11.1%) had ventricular septal defect (VSD), 3/36 (8.3%) had patent ductus arteriosus (PDA), and 2/36 (5.6%) had both ASD and PDA. Nineteen subjects were undernourished and 17 were well-nourished (Table 1).

Using the ANOVA test, we found the optimal cut-off point of TNF- α to be 2,318 pg/mL. Mean TNF- α levels in the CHD-PH, CHD-no PH, no CHD-PH, and no CHD-no PH groups were 2,564.44 (SD 177.00) pg/mL, 2,112.89 (SD 382.00) pg/mL, 2,211.56 (SD 330.70) pg/mL, and 1,118.89 (SD 1056.65) pg/mL, respectively ($p < 0.001$; ANOVA test). The CHD-PH group had the highest mean TNF- α level, followed by the no CHD-PH, CHD-no PH, and no PH-CHD groups (Figure 1). We found that DS children with TNF- α levels $\geq 2,318$ pg/mL had a greater risk of CHD (RR=2.6; 95%CI 1.17 to 5.78; $P=0.008$) and PH (RR=3.5; 95%CI 1.43 to 8.60; $P=0.001$) compared to those with lower TNF- α levels (Tables 2 and 3).

Discussion

Our main finding was that children with DS have an increased risk of PH, which is consistent with other studies. A previous study reported that children with DS had a tenfold risk of PH compared to normal children.¹⁵ The ratio of boys to girls in our study was comparable in all groups, in agreement with epidemiological data reporting the absence of sex difference in DS.¹⁶

The severity of structural abnormalities of the heart and large blood vessels and the resulting hemodynamic disturbances are often associated with feeding difficulties and failure to thrive. This is due to several factors that are frequently present in children with CHD, such as inadequate calorie intake,

Table 1. Baseline characteristics of subjects

Characteristics	Diagnostic classification			
	CHD-PH (n=9)	CHD-no PH (n=9)	No CHD-PH (n=9)	No CHD-no PH (n=9)
Mean age (SD), months	27.00 (19.98)	26.33 (19.60)	29.00 (12.52)	30.11 (17.27)
Gender, n				
Male	6	4	7	4
Female	3	5	2	5
Nutritional status, n				
Well-nourished	4	1	4	8
Undernourished	5	8	5	1
Symptoms, n				
None	0	1	1	8
Tachypnea	0	0	0	1
Cyanosis	1	0	0	0
Difficulty gaining weight	8	8	8	0
Shunt, n				
None	0	0	9	9
VSD	1	3	0	0
ASD	7	2	0	0
PDA	0	3	0	0
ASD and PDA	1	1	0	0

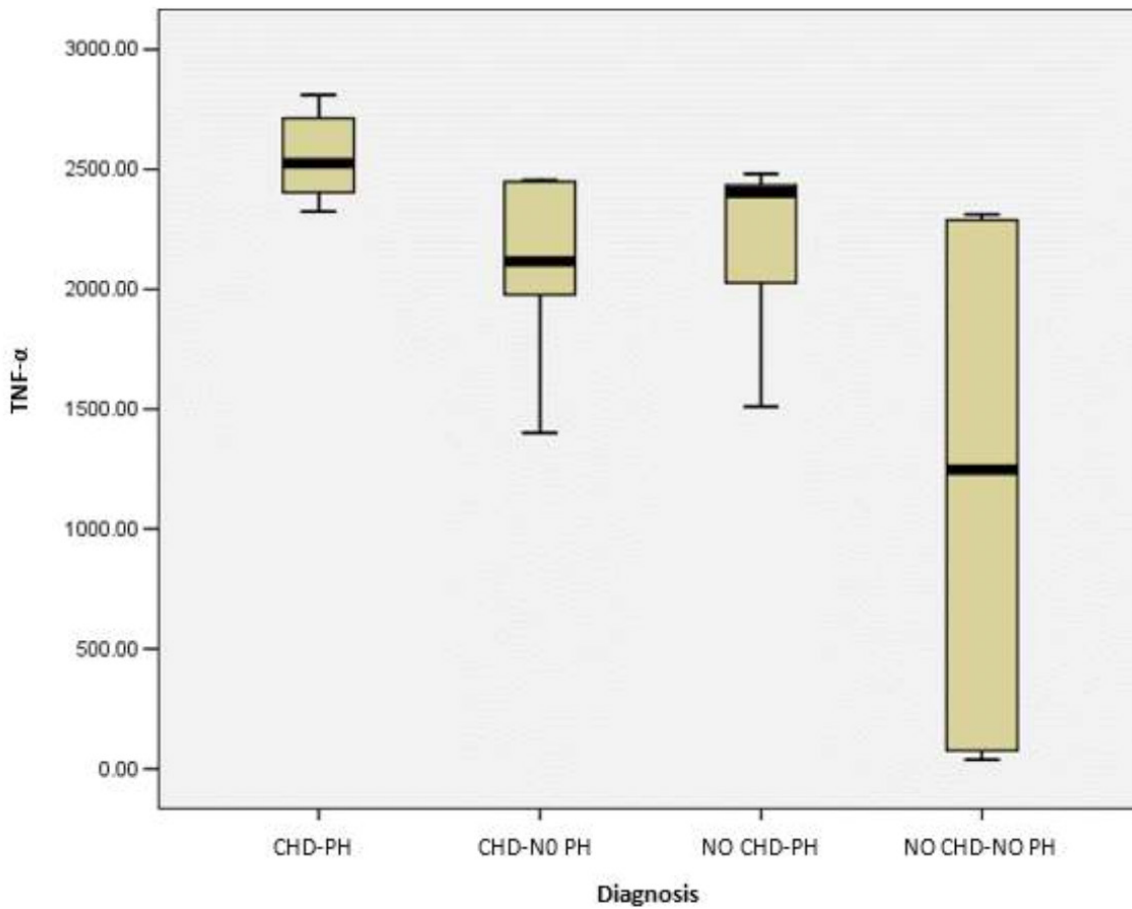


Figure 1. Boxplot of TNF- α level based on the diagnosis

Table 2. The risk of CHD and PH based on TNF- α levels

TNF- α	CHD		Total	RR (95%CI)	P value
	Yes	No			
>2,318	13	5	18	2.60 (1.17 to 5.78)	0.008
<2,318	5	13	18		
Total	18	18	36		

Table 2. The risk of PH risk based on TNF- α levels

TNF- α	PH		Total	RR (95%CI)	P value
	Yes	No			
>2,318	14	4	18	3.50 (1.43 to 8.60)	0.001
<2,318	4	14	18		
Total	18	18	36		

increased oxygen consumption, hypermetabolism, decreased absorption, uneven distribution of nutrients throughout the body, and food intolerance.¹⁷ In our study, 19/36 patients were malnourished. Earlier studies have reported that CHD patients often experience malnutrition and growth problems, and that PH is associated with malnutrition in CHD children.¹⁸⁻²¹ Our findings were consistent with those studies; difficulty gaining weight was one of the initial symptoms of CHD in 66.7% of subjects. For this reason, difficulty gaining weight is one of the symptoms to look for in early screenings for CHD. Data from a major referral hospital in Indonesia showed that the most severe cases of growth faltering in infants born with normal birth weight were due to CHD accompanied by PH.¹⁷

The types of shunts found in our study were ASD (9 subjects), VSD (4 subjects) and PDA (3 subjects). Two previous studies reported that the most common CHD in DS children were ASD, VSD, and PDA, and 53% were accompanied by PH.^{16,22}

Down syndrome children with increased TNF- α levels had a higher risk of PH. We found a significant association between TNF- α and the incidence of CHD as well as PH. DS children with TNF- α \geq 2,318 pg/mL had 2.6 times risk of CHD and 3.5 times risk of PH. A previous study showed in their study on inflammation biomarkers that TNF- α levels of PH patients were higher than those without PH ($P < 0.001$).²³ This finding was due to increased pulmonary vascular resistance resulting from endothelial dysfunction, vasoconstriction, small thrombosis and pulmonary arteries, and remodeling.¹⁷ Similarly, a study also demonstrated that TNF- α levels were higher in PH

cases than controls ($P < 0.001$).²⁴ Therefore, we suggested that DS children with high TNF- α level require screenings for CHD and PH.

Our study had some limitations. It was conducted in a single hospital with a limited number of subjects, which may limit the generalizability of the results. In addition, we did not perform cardiac catheterization, which is considered to be the gold standard in assessing PH; we used echocardiography instead.

In conclusion, children with CHD accompanied by PH have higher TNF- α levels than those without PH and those without CHD. In children with DS, an elevated TNF- α level (\geq 2,318 pg/mL) is associated with a higher risk of CHD and PH.

Conflict of interest

None declared.

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