How to Cite:
Sari, R. K., Rahman, M. A., Prabowo, G. I., Athiyyah, A. F., Endryanto, A., & Oktaviano, Y. H. (2022). Correlation between serum myeloperoxidase with the severity of heart failure in children. *International Journal of Health Sciences*, *6*(S9), 457–465. https://doi.org/10.53730/ijhs.v6nS9.12355

Correlation between serum myeloperoxidase with the severity of heart failure in children

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***Abstract*---**Heart failure in children is a global major health problem. Inflammation is an important role in pathophysiology of heart failure. Myeloperoxidase (MPO) included to the inflammation cascade. The objective was to analyze correlation between MPO with severity of heart failure in children. Observational analytic with cross sectional study was conducted on children aged 1 month-18 years with an acyanotic CHD with or without heart failure at Dr. Soetomo General Hospital Surabaya. Bivariate analysis used Mann Whitney test. Multivariate analysis used Kruskal Wallis. Correlation test used *Spearman's test* with significance value was $p<0.05$. A total of 21 subjects, median age was 93 months old. The most common type of acyanotic CHD was VSD. Moderate heart failure was in 28% of subjects, 48% of subjects were mild heart failure. Median value of

serum MPO levels in acyanotic CHD with heart failure was 36.25 (7.3-390) pg/ml. There was no differences in the value of serum MPO level in children with acyanotic CHD distinguished by the severity of heart failure (p=0.62). There was no correlation between serum MPO levels with the severity of heart failure in children (p=0.89). We concluded no correlation between serum MPO levels with the severity of heart failure in children.

***Keywords*---**myeloperoxidase, heart failure, congenital heart disease, children.

Introduction

Heart failure in children is a global major health problem nowadays (Sibetcheu *et al.*, 2018). Prevalence and incidences of children with heart failure was unclear (Madriago and Silberbach, 2010). Study in UK showed the incidences of children with heart failure was 0.87 in every 100 thousands of children under 16 years diagnosed as cardiomyopathy. Seventy-one percent of children was heart failure (Nandi, Almond, and Rossano, 2017). The etiologies and mechanisms of heart failure in children and adults is significantly different (Blatter, Noyes, and Sweet, 2018). Recent studies suggest the role of inflammation and oxidative stress in the pathophysiology of heart failure. Oxidative stress is an important part in the development of cardiac pathology (Pirinccioglu *et al.*, 2012; Ungvari *et al.*, 2005). A more than 100-fold risk of heart failure was found in children with Congenital Heart Disease (CHD), primarily the acyanotic type of left to right shunt (Gilljam *et al.*, 2019). Chronic inflammation as an important role in the pathophysiology of heart failure, including myocardial remodeling, endothelial dysfunction, and peripheral vascular damage. Massive inflammation causes an imbalance of redox status resulting in many Reactive Oxygen Species (ROS) (Nicholls dan Hazen, 2005; Puggia *et al.*, 2018). Myeloperoxidase (MPO) is an enzyme presenting in leukocytes, part of the ROS cascade. Several studies suggest that increasing of plasma MPO level in patient with heart failure is associated with the worsening of the disease (Tang *et al.*, 2006; Michowitz *et al.*, 2008; Reichlin *et al.*, 2010). The mechanism and role of MPO in children with heart failure has not been widely studied.

Methods

The observational analytic study with cross sectional study design was conducted on children aged one month-18 years with an acyanotic CHD left to right shunt with or without heart failure after written informed consent was given by patient's parent. The protocol of this study was approved by the Ethics Commission of Dr. Soetomo General Hospital (0267/KEPK/IX/2021). The study was conducted at Pediatric Cardiac Center and Pediatric Ward, Department of Child Health, Dr. Soetomo General Hospital Surabaya during October to December 2021.

A total of 21 subjects were recruited in this study. All subjects were calculated the Pediatric Heart Failure Score (PHFS) according to table 1. If the score was 0-2, it means there was no heart failure, if the score was 3-6, it means mild heart

failure, if the score was 7-9, it means moderate heart failure, and if the score was 10-12, it means severe heart failure. Exclusion criteria in this study as follows: (1) children with acyanotic CHD planned for surgery in a month; (2) children with acyanotic CHD with tuberculosis infection, and/or urinary tract infection, and/or pericarditis, myocarditis, endocarditis, and/or septicemia; (3) acute or chronic kidney disease with GFR< 90 ml/minute/1.73 m^2 or with renal replacement therapy; (4) hyperkalemia with potassium level > 5.5 mEq/L or hypokalemia; (5) children with intravenous inotropic drug; (6) children with hyperthyroid; (7) children with cirrhosis hepatic, and/or hepatitis, and/or acute liver failure; (8) children with autoimmune diseases (Systemic Lupus Erythematous, Nephritic Lupus, Juvenile Inflammatory Arthritis, Periodic Fever Syndrome, Dermatomyositis, Scleroderma, and Mixed Connected Tissue Disease); (9) children with vasculitis (Henoch Schonlein Purpura, Kawazaki Disease, and/or Takayasu Disease).

Table 1. Pediatric Heart Failure Score/Modified Ross Score

Clinical Sign and Symptoms	Score		
	0	1	2
Diaphoresis	Only Head	Head and Body while exercise	Head and Body while rest
Tachypnea	Seldom	Sometimes	Often
Work of breathing	Normal	Chest in drawing	Dyspnea
Respiratory Rate (times/minutes)			
*0-1 year	<50	50-60	>60
*1-6 years	<35	35-45	>45
*7-10 years	<25	25-35	>35
*11-14 years	<18	18-28	>28
Heart rate (times/minutes)			
*0-1 year	<160	160-170	>170
*1-6 years	<105	105-115	>115
*7-10 years	<90	90-100	>100
*11-14 years	<80	80-90	>90
Hepatomegaly (liver border below right costae)	<2 cm	2-3cm	>3cm

Source: Ross. 2012. The Ross classification for heart failure in children after 25 years: A review and an age-stratified revision. Pediatr Cardiol.33:1295–300

Sample Collection

Sample was carried out by consecutive sampling, with criteria specified in subjects as mentioned earlier. The sampling took place from October-December 2021, 21 samples were obtained. Samples of blood were taken from vein (5 ml) and collected using vacutainers containing ethylenediaminetetraacetic acid (EDTA).

Myeloperoxidase Analysis

Each blood was separated to use the plasma with the tools such as Micropipette 1000 πL, sterile blue tips, eppendorf, tube, and HC-1180T centrifuge 8 hole. Myeloperoxidase level were determined quantitatively by sandwich-type enzyme-linked immunosorbent assay (ELISA) kit based on manufacture protocol of *abbexa®, Human Myeloperoxidase ELISA Kit*.

Statistical Analysis

Normality Data was analyzed by Saphiro-Wilk test. The comparison of MPO level and severity of heart failure were analyzed by Mann Whitney U-test for the bivariate analysis and by Kruskal Wallis test for the multivariate analysis with P< 0.05. The correlation between MPO level and severity of heart failure were analyzed by Spearman's rho. Data analysis using SPSS for Windows version 16 (SPSS Inc., Chicago, Illinois, USA).

Results and Discussion

In this present study, a total of 21 subjects met inclusion criteria, with 71.4% was girl. Characteristics data of the samples was written below at table 2.

Table 2. Characteristics data of subject

Characteristics	n = 21
Age (month), median (min-max)	93 (13-213)
Age categories (month), n (%)	
0-12	0
13-83	10 (47.6)
84-131	4 (19)
>131	7 (33.3)
Gender, n (%)	
Boy	6 (28.6)
Girl	15 (71.4)
Type of Acyanotic CHD, n (%)	
VSD	8 (38.1)
ASD	4 (19)
PDA	6 (28.6)
ASD and PDA	2 (9.5)
VSD and ASD	1 (4.8)
Nutritional status, n (%)	
Well nourished	3 (14.3)
Moderate malnutrition (wasted)	9 (42.9)
Severe malnutrition (severely wasted)	3 (14.3)
Overweight	1 (4.8)
Short stature	5 (23.8)
Hepatomegaly, n (%)	
Yes	12 (57.1)
No	9 (42.9)
Heart failure based on PHFS, n (%)	

No	5 (23.8)
Yes	16 (76.2)
Mild heart failure	10 (47.6)
Moderate heart failure	6 (28.6)

VSD: ventricle septal defect, ASD: atrial septal defect, PDA: patent ductus arteriosus

The most common type of left to right shunt acyanotic CHD was VSD, followed by PDA and ASD. There were 42.9% subjects in moderate malnutrition and 1 subject was overweight. Moderate heart failure was in 28% of subjects and 48% of subjects were mild heart failure. There were 23.8% of subjects without heart failure.

In this present study, we investigated the comparison between MPO level and the presentation of heart failure in children with acyanotic CHD. The result was presented in table 3.

Table 3. The comparison between MPO serum level in children with acyanotic CHD and heart failure and children with acyanotic CHD without heart failure

Criteria	Median MPO (min-max) (pg/ml)	Total (n)	P
Heart failure	36.25 (7.3-390)	16	
No heart failure	27.6 (3.3-68,4)	5	0,483*

*value are no differences p>0.05, according to Mann Whitney U test.

The median value of MPO serum in children with acyanotic CHD without heart failure was 27.6 pg/ml and median value of MPO serum in children with acyanotic CHD and heart failure was 36.25 pg/ml. The statistical result revealed for the comparison between MPO serum level in children with acyanotic CHD with and without heart failure was no differences. The severity of heart failure was analyzed to compare each degrees of severity with the level of MPO serum in children with acyanotic CHD. The result was presented in table 4.

Table 4. The comparison between MPO serum levels in children with the severity of heart failure

Criteria	Median MPO (min-maks)(pg/ml)	Jumlah (n)	P
No heart failure	27.6 (3.3-68.4)	5	
Mild heart failure	45.5 (7.3-379.4)	10	0,620*
Moderate heart failure	21(8.1-390)	6	

*value are no differences p>0.05, according to Kruskal Wallis test

In this present study, there was no children with severe heart failure. After analyze the comparison, we investigated the correlation between serum MPO levels with the severity of heart failure in children with p value 0.89 and coefficient correlation was 0.033. The statistical result showed there was no correlation.

Discussion

Inflammation had an important role in pathophysiology of heart failure, in response to cardiac injuries which was the first process of heart failure presentation (Puggia *et al.*, 2018). There was 71.4% was girl with median age was 93 months. Other study in Dr. Soetomo General Hospital Surabaya 2021 revealed girl was dominant in children with acyanotic CHD and heart failure, which was 53.5% (Arifani, Rahman, and Nugraha, 2021). In line with other study in Indonesia 2016 stated the proportion of girl in children with heart failure was higher, 66.67% (Mahrani, et al., 2017). This present study showed most common acyanotic CHD in children with heart failure was VSD, 38.1%, followed by PDA (28.6%), and ASD (19%). This condition was in line with study in Palembang and Surabaya (Arifani, Rahman, and Nugraha, 2021; Mahrani, et al., 2017). In the other hand, epidemiology data in China 2020 stated that most common acyanotic CHD in children was ASD, followed by PDA and VSD (Zhang *et al.*, 2022).

The median value of MPO serum in children with acyanotic CHD and heart failure was 36.25 pg/ml. Recent study about children with heart failure and rheumatic heart disease revealed that mean MPO serum was 126.13 ±24 pg/ml (Putri *et al.*, 2017). Preclinical study in 2016 stated that administration of MPO inhibitor in mice could increase ventricular function and repair the remodeling of cardiomyocytes (Ali *et al.*, 2016). Study in Egypt 2019 about the significances of MPO levels with incidence of cardiovascular diseases and obesity, revealed there was no differences activity of MPO serum in control and children with obesity and risk of cardiovascular disease (Alameey *et al.*, 2019). Other study in pre-puberty children aged 6-12 years, there were increasing of MPO levels in children with obesity. This study compared between children with normal nutritional status and with obesity. The increasing of MPO enzyme correlated with risk factor of cardiovascular and inflammation (Olza *et al.*, 2012). Cohort study performed in adult patients revealed that there was increasing of plasma MPO levels in adults with chronic heart failure. Increasing of plasma MPO levels was 1158±2965 pg/ml compared with controls which was 204±139 pg/ml, $p<0.0001$ (Tang *et al.*, 2006). Other study stated that MPO levels was higher in patients with congestive heart failure than healthy subjects, which was 205.7±272,6 ng/ml and 123±170,5 ng/ml (Michowitz *et al.*, 2008). Experimental study stated that MPO and oxidant derived MPO (hypochlorite) affected fibronectin formation. Fibronectin is specific matrix protein which is secreted by immune cells and myofibroblast. Fibronectin caused fibrosis formation in smooth muscles and cardiomyocytes so that fibrosis was developed (Nybo *et al.*, 2018). However, study about MPO level in children with acyanotic CHD and heart failure is still limited.

In this present study, there was no correlation between serum MPO levels with the severity of heart failure in children with acyanotic CHD. This study was first study which analyze the correlation between serum MPO levels with the severity of heart failure in children with acyanotic CHD. Recent study was conducted in adult patients, which was the meta-analysis study in 2019. The result showed there was correlation between the high levels of MPO serum with the risk factor of mortality in people with heart failure. Myeloperoxidase values could be employed as risk stratification model in therapy administered to patient with high risk of acute coronary syndrome (Kolodziej *et al.*, 2019). Other study stated that there

was correlation between the increasing levels of MPO plasma with the severity of heart failure based on New York Heart Association Class and B-type Natriuretic Peptide plasma (Tang *et al.*, 2006). Study by Tang in the next year stated that MPO levels could be used as predictor values in long-term outcome of patient with heart failure (Tang *et al.*, 2007). Recent study from Tang stated that the increasing levels of MPO and hsCRP increases risk of heart failure six times (Tang *et al.*, 2011). Study from Romania analyzed levels of MPO serum in adults with diastolic dysfunction. Myeloperoxidase values independently correlated with the results of echocardiography which was diastolic dysfunction (Coculescu *et al.*, 2018). The drawbacks of this study was no prior data of MPO level in subjects before they fell in heart failure condition.

Conclusion

In conclusion, the present study demonstrated there was no differences and no correlation between the values of MPO serum with the severity of heart failure in children with acyanotic CHD. Despite there was no differences, the inflammation may still occurred in the presentation of heart failure. The result study suggests further study about severity of heart failure in specific type of acyanotic CHD and also add on the others marker of inflammation.

Acknowledgements

The authors are grateful to all the patients, nurses, laboratory analyst, and others who support this present study. None of the authors has a commercial association, such as consultancies, stock ownership or other equity interests or patent-licensing arrangements.

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