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Antioxidant Status in Congenital Heart Disease Children with Heart Failure

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ABSTRACT

Heart failure is major contributor to morbidity and mortality in children with congenital heart disease (CHD). Recently heart failure's progression is often associated with oxidative stress. Superoxide dismutase (SOD) is first line antioxidant of defense against superoxide anion. Catalase (CAT) breaks down hydrogen peroxide into water and oxygen molecules which complements previous detoxification carried out by SOD. The objective of the study is to compare the differences of SOD and CAT levels in acyanotic CHD patients between those with and without heart failure. A case-control study was conducted on three to ten years old children with a left-to-right shunt acyanotic CHD with and without heart failure in the Pediatric Cardiology outpatient clinic, ward, and emergency room of Dr. Soetomo Hospital Surabaya from March-June 2023. Echocardiography was used to establish the CHD, while Pediatric Heart Failure Score (PHFS) criteria was used to asses heart failure. T-test was undertaken for analyzing the difference between both groups. The total samples were 41 children, consisted of 29 subjects in the case group (CHD with heart failure) and 12 subjects in the control group (without heart failure). The level of SOD in CHD with heart failure was lower (74.670+15.705) than those without it (109.163+3.111) ($p<0.05$). In contrast, CAT level in CHD with heart failure was higher (25.895) than those without it (13.976) ($p<0.05$). There was a significant difference of SOD and CAT levels in acyanotic CHD between those with and without heart failure.

Keywords: Acyanotic CHD; Antioxidant; CAT; Children; Heart failure; Oxidative stress; SOD

INTRODUCTION

The most common complication in CHD is heart failure (HF) that closely relates to ventricular dysfunction and overload of volume and pressure[1]. It is most commonly attributable to coexistent CHD, with different risks depending on the specific type of malformation[2]. The incidence of HF in CHD is about 0.8% of live births[3]. There are about 12,000-35,000 cases of HF that causes about 11,000-14,000 cases of hospitalization for children aged less than 19 years in the United States each year[4]. Heart failure is defined as a clinical syndrome caused by either inability to pump enough blood throughout the body as needed or unable to return pulmonary or systemic blood flow or the combination of both[5]. It is a result of disorders of the structure and function of the heart that affect the ability of the ventricles to fill and/or pump blood[6]. Nandi and Rossano stated that HF, regardless of the etiology, is a syndrome of clinical and pathophysiological symptoms caused by impaired filling or blood pump in the ventricles that causes inadequate organ perfusion[7]. It is a complex disease process that can occur secondary to various underlying etiologies, such as CHD, cardiomyopathy, and/or other acute conditions[8,9].

Until now, the definitive diagnosis and therapy of HF in children with CHD remain unsatisfactory. Recently, the progression of HF in CHD has been associated with oxidative stress process approach. Moreover, increasing production of reactive oxygen species (ROS) is associated with the emergence of both pulmonary and systemic vascular disorders resulting in HF[10]. Superoxide Dismutase (SOD) and Catalase (CAT) are type of antioxidant produced in the mitochondria of heart muscle that work against excessive ROS. Basic knowledge about the levels of SOD and CAT to determine the oxidative stress process that occurs in HF as a basis for innovative diagnosis and therapy of HF in CHD patients in the future is necessary[11]. This study aimed to compare the differences of SOD and CAT levels in acyanotic CHD patients between those with and without HF.

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MATERIALS AND METHODS

This study was an analytical observational research with a case-control design to compare SOD and CAT levels in left-to-right shunt acyanotic CHD between those with heart failure and without heart failure. The samples were all children aged 3-10 years old with a left-to-right shunt acyanotic CHD with heart failure and those without heart failure who came through Emergency room (ER) and the Pediatric Cardiology Outpatient Clinic (OPC) in the period after obtaining permission and approval from the Health Research Ethics Committee at Dr. Soetomo Hospital Surabaya. Informed consent was obtained from all individual participants included in the study. They were chosen using consecutive non-random sampling. The definitive diagnosis of acyanotic CHD was determined based on echocardiographic examination by pediatric cardiology consultant. Types of heart defects categorized as CHD acyanotic left-to-right shunt here were ventricular septal defect (VSD), atrial septal defect (ASD), and patent ductus arteriosus (PDA). While the diagnosis of heart failure was established by Pediatric Heart Failure Score (PHFS) criteria.

Inclusion criteria were children with left-to-right shunt acyanotic CHD aged 3-10 years old who meet the clinical criteria for heart failure according to PHFS with a score more than 2 for the case group. While the exclusion criteria were children with left-to-right shunt acyanotic CHD who had cardiac surgery plan within the next one month, impaired kidney function with decreased Glomerular Filtration Rate (GFR) according to age or with renal replacement therapy, malignancy, hyperkalemia with potassium levels more than 5.5 mEq/L or hypokalemia with potassium levels less than 3 mEq/L, history of metabolic and hormonal diseases (e.g. diabetes mellitus, hypothyroidism, hyperthyroidism, and congenital adrenal hyperplasia), girls who had menstruated, diseases affecting the vascular (e.g vasculitis, hypertension, and cerebrovascular disease), and critically-ill (getting intravenous inotropes, pneumonia on a ventilator, terminal conditions, and sepsis).

Tool to check the level of SOD from blood was the Superoxide Dismutase Typed Activity Kit (Elabscience paint # E-BCK022-S). Sampling was 1 ml venous blood plus citrate/EDTA, then centrifuged for 10 minutes at 40^0C. Plasma layer was transferred to a new tube and stored at 80^0C then analyzed with a 450 nm ELISA kit microplate reader. The level of SOD was expressed in units of U/ml. The HUMAN Catalase assay Kit (Cayman Chem. Paint # 707002) was used to check CAT level. Sampling was 0.2 ml erythrocytes in 0.2 ml cold assay buffer then centrifuged for 15 minutes at 4^0C. The supernatant was taken and kept in ice, measured by the calorimetric method at 570 nm. Level of CAT was expressed in μM units.

All data were analyzed using SPSS version 20.0. The basic characteristics of research subjects (e.g. age, gender, nutritional status, and type of CHD) were presented descriptively in tabular form. To find out whether there were differences in SOD and CAT levels between the case and control groups, the data normality test was firstly conducted with Kolomogorov-Smirnov test. If the data were normally distributed ($p>0.05$), the data were tested with an independent sample T-test, but if not ($p<0.05$), the data were tested using a Mann-Whitney test.

The ethical clearance was approved by the Ethical Committee of Dr. Soetomo General Hospital Surabaya.

RESULTS

Initial sampling was conducted from March to June 2023. During the study periods, there were eight subjects resigned because their parents refused to sign an informed consent. Therefore, at the end of the study, the total sample that met the inclusion criteria was 41 subjects, consisted of 29 subjects in the case group (CHD with heart failure) and 12 subjects in the control group (CHD without heart failure). Descriptive analysis was carried out to see the frequency and distribution of variables. Table 1 shows the characteristics of children.

Table 1: Characteristics of samples

Variables	Percentage (%)		p
	CHD with Heart Failure (N = 29)	CHD without Heart Failure (N = 12)	
Sex			
Male	13 (45%)	6 (50%)	0.763
Female	16 (55%)	6 (50%)	
Median age (years)	9 (2-14)	8.5 (2-14)	0.806
Nutritional Status			
Normal	24 (83%)	12 (100%)	0.125
Moderate	5 (17%)	0 (0%)	
Type of CHD			
VSD	17 (59%)	6 (50%)	0.113
PDA	12 (41%)	3 (25%)	
ASD	0	3	

Table 2: The level of SOD dan CAT between CHD accompanied by heart failure and without heart failure

Variables		Acyanotic CHD		p
		with heart failure (N = 29)	without heart failure (N = 12)	
Mean (U/mL)	SOD	74.670 (+ 15.705)	109.163 (+ 3.111)	0.001*
Median (μM)	CAT	25.895 (6.070-70.230)	13.976 (10.430-16.419)	0.002**

*) Independent sample T-test
**) Mann-Whitney test

There was no statistically significant difference in the proportion of sex between the two groups although the number of girls in case group was slightly higher than those in control group. In the case and control groups, the median age values were 9 and 8.5 years, respectively. There was no statistically significant difference in the age variable between the two groups. Most of the subjects in both groups were still in normal nutritional status. Ventricular Septal Defect (VSD) was still the most common type of CHD in both groups.

In the SOD variable, after the normality test was carried out with Kolomogorov-Smirnov test, it was found that the data were normally distributed in both groups ($p > 0.05$), therefore the numerical data were presented in the form of a mean accompanied by a standard deviation value. The mean SOD levels in CHD patients with heart failure were lower (74,670 + 15,705) than those without heart failure (109.163 + 3.111). The data were then analyzed by parametric test using independent sample T-test. As a result, there was a significant difference in SOD levels in the two groups ($p<0.05$).

On the contrary, the CAT variable data were not normally distributed ($p<0.05$), therefore the numerical data were presented in the form of the median accompanied by the maximum and minimum values. The median CAT results in patients with CHD with heart failure were higher (25.895) than those without heart failure (13.976). The data were then analyzed by non-parametric test using the Mann-Whitney. As a result, there was also a significant difference in CAT levels in the two groups ($p<0.05$).

DISCUSSION

In this study, SOD level in children with acyanotic left-to-right shunts with heart failure was lower than those without heart failure. Superoxide dismutase is basically the first line of antioxidant defense against the process of oxidative stress formation [11]. This enzyme has an important role in superoxide metabolism because of its closest location to the main source of producing ROS, mitochondria [12]. If there is a decrease in SOD levels, this oxidative stress activity will cause a series of processes that lead to endothelial damage and remodeling of the heart muscle then ultimately will lead to heart failure. Moreover, recent evidence suggests that at each subcellular location, SODs catalyze the conversion of $O_2^{\cdot-}$ and H_2O_2 that have a role in cell communication. It also plays an important role in inhibiting the oxidative inactivation of NO thus preventing peroxynitrit formation and also endothelial and mitochondrial dysfunction. In normal cellular metabolic processes, oxygen undergoes a series of univalent reductions that cause the formation of $O_2^{\cdot}$, hydrogen peroxide (H_2O_2), and H_2O. Potential sources of ROS-forming enzymes include components of the electron transport chain, xanthine oxidase, cytochrome p450 monooxygenase, lipoxygenase, NOS, and NADPH oxidase. Anion superoxide is dismutated by SOD into H_2O_2 and then catalyzed into H_2O by the enzyme catalase, peroxiredoxins (Prxs), or glutathione peroxidase (GPx) [13,14].

The result of this study is consistent with a case control study conducted by Gao-Zhong et al [13] in 2010 for children aged 1 month to 3 years with left-to-right shunt acyanotic CHD. The level of SOD with heart failure was found to be lower than the control group ($p <0.01$). Gao-Zhong concluded that the process of oxidative stress was the cause of incidence of left-to-right shunt CHD accompanied by heart failure [13].

The result of this study is also in accordance with a study conducted by Lu et al in 2008 which aimed to determine SOD levels in heart failure conditions conducted on experimental animals. The results of this study indicate that there is a significant decrease in SOD1 levels in mice after transverse aortic constriction (TAC) action which may partially contribute to the heart failure and left ventricle remodelling. This result concludes that oxidative stress has been shown to interfere with mitochondrial metabolism and cardiac muscle contractility function. This study provides direct evidence that extracellular SOD possibly has an important role in protecting the heart against oxidative stress-induced overload and impaired contractility function. Although there is evidence of increased production of free radicals in heart failure, other evidence also suggests that decreased antioxidant stores contribute to increased oxidative stress in several models of myocardial dysfunction [15].

On the contrary, CAT levels in children with left-to-right acyanotic CHD who experienced heart failure in the present study were found to be higher than those without heart failure. Unlike SOD, Catalase enzymes are not the first line of antioxidant enzymes to fight ROS. This enzyme uses iron or manganese as a cofactor and serves to catalyze the degradation or reduction of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2) molecules that will complement the previous detoxification process carried out by SOD. This enzyme works effectively by breaking down millions of hydrogen peroxide molecules in seconds [14]. In carrying out its function, CAT is assisted by the enzyme Glutathione Peroxidase (GPx) and Peroxiredoxin. Based on its chemical characteristic, GPx has a much higher affinity ability to bind hydrogen peroxide compared to CAT [16]. This may be the possible explanation why CAT levels in patients with left-to-right shunt acyanotic CHD who experienced heart failure in this study remained higher than those without heart failure. However, in this study, the levels of GPx and Peroxiredoxin were not measured.

The production of free radical molecules, such as $O_2^{\cdot-}$ and H_2O_2 can cause several mechanisms. Activation of redox sensitive kinase which will cause inflammation and angiogenesis. Inactivation of protein phosphatase to modulate redox-sensitive signaling will lead to hypertrophy and proliferation of endothelial cells. Activation of redox-sensitive transcription factors will cause proinflammatory

gene expression (VCAM1, MCP1). Modulate calcium ion channels (Ca^{2+}) have an effect on vascular remodeling, inflammation, increasing vascular permeability, and angiogenesis. Next, activation of matrix metalloproteinases (MMPs) will cause extracellular matrix remodeling. Apart from $O_2^{\cdot-}$ and H_2O_2, nitric oxide synthase (NOS) has a role in the pathophysiological mechanisms of heart disease. The formation of NOS begins with the nitric oxide (NO) molecule that quickly reacts with $O_2^{\cdot-}$ with the help of the enzymes NADPH oxidase, xanthine oxidase, and mitochondria to form peroxynitrite anion ($ONOO^-$), of which will oxidize several molecules, including heme from soluble guanylate cyclase (sGC), lipids, and endothelial NOS (eNOS). These reactions will result in vascular inflammation, changes in vascular tone, increasing vascular permeability and platelet aggregation. Vascular superoxide ($O_2^{\cdot-}$) is normally produced by the product of cellular metabolism and will differ it to form other types of ROS, such as hydrogen peroxide (H_2O_2) dan Peroxynitrite ($ONOO^-$). In blood vessels, changes in superoxide levels will affect vascular tone modulation, gene expression, inflammation, cell growth, signaling, and apoptosis. Superoxide dismutase (SOD) as the first line against ROS in living cells will catalyze the reduction and oxidation reactions from superoxide to hydrogen peroxide and oxygen molecules. If there are reduced transition metals (Fe^{2+}, Cu^{2+}), H_2O_2 can undergo a spontaneous conversion to hydroxyl radicals ($OH^{\cdot}$) or other types of ROS that are highly reactive. Nitric oxide (anti-inflammatory, anticoagulant, and vasodilator) can also be inactivated very quickly when it reacts with $O_2^{\cdot-}$ and causes the production of the strong oxidant peroxynitrite ($ONOO^-$) and endothelial and mitochondrial dysfunction. Therefore, SOD is the first line of defense against the toxicity of anionic superoxide radicals. SOD activity requires metal catalysis [11].

Furthermore, excessive ROS induces proliferation of myocardial fibroblasts by remodeling of cardiac muscle. Experimental results in animals with heart failure led to the hypothesis that oxidative stress could be a further therapeutic target in heart failure patients. Clinical trials have begun to study the effects of antioxidant therapy in humans with heart failure. Treatment of oxidative stress itself can have different approaches, namely inhibition of oxidative stress producers, increasing endogenous antioxidant capacity, and increasing antioxidant capacity by administering exogenous antioxidants, such as vitamin C, vitamin A, vitamin E, and folic acid [17].

Research Limitations

Case-control study is observational and does not provide the same level of evidence as randomized controlled trials and meta-analyzes. The effect of external variables is technically difficult to control and measure due to the large number of confounding factors, for example hormonal factors and endogenous antioxidant content (e.g. vitamins A, C, and E) or nonenzymatic components including glutathione, metallothionein, a-tocopherol, and ascorbate. Heart failure is a complex disorder. There are many compensatory mechanisms for overcoming inadequate perfusion of vital organs. Impaired local circulation of important organs along with endothelial and neurohormonal dysfunction are associated with oxidant-antioxidant imbalance and subcellular disorders.

Research Strength

The present study is one of the few studies revealing the differences of SOD levels in pediatric patients with CHD who experienced heart failure. Previous studies had been more frequently conducted and focused on experimental animals and adult.

CONCLUSION

There is a significant difference of SOD and CAT levels in a left to right shunt acyanotic CHD between those with heart failure and without it. The level of SOD in children with heart failure is lower than those without heart failure. While CAT level in children with heart failure is higher than those without heart failure. The process of oxidative stress is the ultimate cause of incidence of heart failure in children with left-to-right shunt CHD.

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