A Preliminary Study: Troponin T and Reg3β in Children with Left-to-Right Shunt Congenital Heart Disease with Heart Failure

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Abstract

Background: Congenital heart disease (CHD) cause heart failure and myocardial injury. Troponin in the heart is a biomarker of myocardial injury in adults and children. Studies that examine troponin T and Reg3β in children with left-to-right shunt CHD with heart failure are still limited.

Objective: This study aims to analyse the troponin T and Reg3β in children with left-to-right shunt CHD with heart failure compared to children without heart failure.

Method: This study was a case control study of children with left-to-right shunt CHD with heart failure and children with left-to-right shunt CHD without heart failure performed with non-random sampling consecutive techniques at the Dr. Soetomo General Hospital, Surabaya in April–June 2019. The diagnosis of left-to-right shunt CHD was determined based on echocardiographic examination. All subjects with left-to-right shunt CHD were evaluated using the Paediatric Heart Failure Score. Troponin T examination was carried out using a one-dimensional electrophoresis technique, which is 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Reg3β examination was carried out by the ELISA method. Data analysis was performed with an independent sample t test using the SPSS.

Results: This study involved 11 children, consisting of 7 children with left-to-right shunt CHD with heart failure and 4 children with left-to-right shunt CHD without heart failure. Most children (72.7%) were female, 3 children (27.3%) were ≤ 5 years old, 5 children (45.4%) were 5–10 years old and 3 children (27.3%) were > 10 years old. There was a significant increase in the Troponin T and Reg3β in children with left-to-right shunt CHD with heart failure as compared to children without heart failure.

Conclusion: Troponin T and Reg3β can be used as biomarkers in children with left-to-right shunt CHD with heart failure.

Keywords: *Congenital Heart Disease, Troponin T, Reg3β, Heart Failure.*

Introduction

Congenital heart disease (CHD) is a congenital disorder that often occurs in children[1]. Excessive pressure and volume in children with CHD creates the risk of injury to the myocardium[2]. Troponin in the heart is a biomarker of myocardial injury in adults[3]. Injury to the myocardium is the cause of elevated troponin levels in 60% of cases[4]. Studies in children mention the role of troponin as a diagnostic marker of myocarditis in children[5].

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Injury to the myocardium raises the body's response by triggering proliferation, phagocytosis and M2 macrophage polarization[6]. This role is mediated by Reg3β, which is increased due to cardiac inflammation[7] and provides a protective mechanism when cardiac injury and stress occur[6]. Previous studies have mentioned an increase in the level of injured Reg3β[8].

Several studies of biomarkers of myocardial injury have been carried out to predict the prognosis and improve outcomes in patients. Troponin T is associated with the degree of myocardial damage and can predict morbidity and mortality due to heart disease [9]. Troponin T is associated with prognosis and mortality in heart failure [10]. Another study stated that Reg3β can be used as a prognostic factor in mortality in patients with acute coronary syndrome [7]. Studies that examine troponin T and Reg3β in children with left-to-right shunt CHD with heart failure are still limited.

Material and Method

This research was a case control study conducted at the Paediatric Cardiology Outpatient Clinic, Emergency Room and Paediatric Ward, Dr. Soetomo General Hospital, Surabaya in April–June 2019. Subjects were children with left-to-right shunt CHD with heart failure with a comparison group, children without heart failure. The diagnosis of left-to-right shunt CHD was determined by echocardiographic examination. The types of cardiac abnormalities categorized as left-to-right shunt CHD included ventricular septal defect (VSD), atrial septal defect (ASD) and patent ductus arteriosus (PDA). Inclusion criteria in this study were age between 5 and 10 years and meeting the clinical criteria for heart failure according to the Paediatric Heart Failure Score. Exclusion criteria in this study were children who scheduled for surgery within the next month, impaired renal function, hyperkalaemia with serum potassium levels > 5.5 mEq/L and unstable clinical conditions, such as receiving intravenous inotropes, ventilator pneumonia and sepsis. Sampling was done by consecutive non-random sampling techniques. All subjects with left-to-right shunt CHD were evaluated using the Paediatric Heart Failure Score.

Troponin T measurement was carried out using a one-dimensional electrophoresis technique, which is 12% SDS-PAGE. Reg3β measurement was performed using the ELISA method.

The mean difference between the two groups was evaluated by the independent sample t test if the data were normally distributed and the Mann-Whitney U test if the data were not normally distributed. The normality of data distribution was tested with the Shapiro-Wilk test. Differences in troponin T and Reg3β in children with left-to-right shunt CHD with heart failure and children without heart failure were analysed using an independent sample t test. Data were analysed using the Statistical Package for Social Sciences (SPSS).

Findings: A total of 11 children were involved in this study, consisting of 8 (72.7 %) females, 3 children (27.3 %) ≤ 5 years old, 5 children (45.4 %) 5–10 years old and 3 children (27.3 %) > 10 years old (Table 1).

Table 1. Subject Characteristics

Variable	Case n (%)	Control n (%)
Sex		
Female	5 (71.4 %)	3 (75 %)
Male	2 (28.6 %)	1 (25 %)
Age		
≤ 5 years old	2 (28.6 %)	1 (25 %)
5–10 years old	3 (42.8 %)	2 (50 %)
> 10 years old	2 (28.6 %)	1 (25 %)

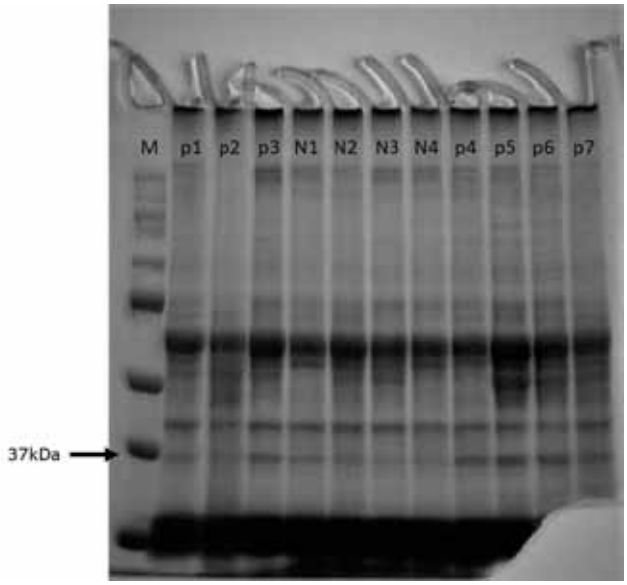

Figure 1. Troponin T

Figure 1 showed the measurement of Troponin T using 12% SDS-PAGE. This study showed that there was a significant increase in 37 kDa protein distribution (Troponin T). The protein band profile obtained from the SDS-PAGE electrophoresis showed differences in

the synthesized protein bands. The group with left-to-right shunt CHD with heart failure had higher Troponin T levels as compared to the group without heart failure (Table 2).

Table 2. Level of Reg3β and Troponin T

	Case (Mean ± Standard Deviation)	Control (Mean ± Standard Deviation)
Reg3β (ng/mL)	19.063 ± 0.619	9.978 ± 0.678
Troponin T	42.600 ± 15.545	6.831 ± 5.61

Reg3β ELISA results showed that there was a significant increase in Reg3β levels in the left-to-right shunt CHD group with heart failure as compared to controls (Table 2).

Discussion

CHD is a congenital disorder that often occurs in children and accounts for up to 1/3 of cases of congenital abnormalities in children [1]. CHD occurs in 6 to 10 per 1000 live births, with an average of 8 out of 1000 live births [11]. The highest prevalence of CHD occurs in Asian countries, which is 9.3 out of 1000 live births [1]. CHD is divided into several types, including right-to-left shunt CHD, obstructive heart disease, cyanotic heart disease and miscellaneous heart disease. The pathogenesis of CHD is multifactorial, including genetic factors or chromosomal abnormalities, environmental factors, maternal infections, smoking and alcohol during pregnancy, pregnancy with diabetes mellitus and obesity during pregnancy or interactions of all these factors [12].

Heart failure can be a complication of CHD. Left-to-right shunts of CHD that can generally cause heart failure include VSD, ASD, atrioventricular septal defect (AVSD) and patent ductus arteriosus (PDA) with moderate to large diameter defects. Children with CHD have a risk of myocardial injury due to excess pressure and volume [2].

Troponin is a single homogeneous protein consisting of four main protein fractions using SDS-PAGE, namely fraction 2, fraction 3 and fraction 4 [13]. There are three forms of troponin, namely troponin T (TnT), troponin I (TnI) and troponin C (TnC) [14]. Troponins in the heart are distinguished by regions with different amino acid sequences. Fraction 2 (~24 kDa) is called TnI ('I' for inhibitory), which inhibits the activity of Mg^{2+}-dependent actomyosin ATPase in the absence of Ca^{2+}. Fraction 3 (~37 kDa) is bound to tropomyosin, so it is called TnT ('T' for tropomyosin), which connects tropomyosin and troponin complexes. Fraction 4 (~20 kDa) is bound to Ca^{2+} and is referred to as TnC ('C' for calcium), which regulates the activity of thin-filaments [13].

Troponin and tropomyosin work to regulate muscle contraction [14]. Troponin levels increase significantly in children with VSD and ASD as compared to healthy children. This condition indicates a significant increase in volume and pressure because left-to-right shunts in CHD can cause damage to the myocardium [15]. Troponin correlates with oxygen saturation and ejection fraction in children with CHD [2].

This study found that children with left-to-right shunt CHD with heart failure had higher levels of troponin T as compared to children without heart failure. Troponin T is associated with myocardial injury in children. However, studies in new-borns have shown that troponin T levels were neither related to the type of heart failure nor the type of heart abnormality [16].

Reg (Regenerating gene) is a protein that was first isolated from rat cDNA and consists of three subtypes, namely types I, II and III. Reg3 consists of Reg3α, Reg3β and Reg3γ [17]. Reg3β was first found in mice models with pancreatitis [18]. Previous studies have suggested that Reg3β was associated with the intensity of inflammation in the heart and increased in cases of acute coronary syndrome [7].

In this study, there was an increase in Reg3β levels in children with left-to-right shunt CHD with heart failure. Previous studies have suggested that Reg3β was involved in the mechanism of protection against cardiac injury and stress [6]. Reg3β levels increase in cardiac ischemia. Healing myocardium after injury requires macrophages [8]. Reg3β plays a role in the repair of myocardial injury by triggering proliferation, phagocytosis and polarization of M2 macrophages [6].

Conclusion

Troponin T and Reg3β can be used as biomarkers in children with left-to-right shunt CHD with heart failure.

Conflict of Interest: The authors declare that there is no conflict of interest regarding this research.

Source of Funding: The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical Clearance: This study was approved by the Ethical Committee of Dr. Soetomo General Hospital, Surabaya No. 1198/KEPK/V/2019.

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