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Prominently Increased of Mannose-Binding Lectin (MBL) and Myeloperoxidase (MPO) Levels in Severe Valve Regurgitation and Heart Failure of Rheumatic Heart Disease

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ABSTRACT

Rheumatic heart disease (RHD) is mediated by an abnormal immunological response following a Streptococcus pyogenes infection that induces a disturbance of oxidants and antioxidants balances. Mannose-binding lectin (MBL) binds to N-acetylglucosamine, a molecule present on the *Streptococcus* cell wall and human heart valves. There is a disturbance of oxidant and antioxidant balance in rheumatic disease. Myeloperoxidase (MPO) is a marker of oxidative stress and inflammation. This study was aimed to determine the correlation of MBL and MPO levels and severity of valvular regurgitation and heart failure (HF) in RHD patients. A case-control study was conduct using human peripheral blood samples from 32 children aged 6 to 14 years old. The subjects were divided into two groups: 16 RHD patients included in the case group and 16 healthy children as a control group. The level of MBL and MPO was investigated using ELISA method. There were significant differences on MBL and MPO level between patient and control group. The level of MBL and MPO were significantly increased in RHD group, especially on severe valvular regurgitation. There was a strong correlation between MBL and MPO levels and the severity of valvular regurgitation (r = 0.94 and r = 0.88). The least significant difference (LSD) analysis showed that significant difference occurs in the severe heart failure group. Our research revealed that the MBL and MPO levels in pediatric RHD patients were significantly higher than in healthy children. The MBL and MPO levels were significantly correlated with the severity of valvular regurgitation and heart failure.

Keywords: *Rheumatic heart disease, valvular regurgitation, heart failure, mannose-binding lectin (MBL), myeloperoxidase (MPO)*

INTRODUCTION

Rheumatic heart disease is a sequela of acute rheumatic fever (ARF) caused by group A streptococci resulting in heart valve damage and can lead to heart failure in severe conditions. Mannose-binding lectin (MBL) binds to N-acetyl glucosamine (GIcNAC), a molecule found on the walls of Streptococcus and also be found on the human heart valves [1].

Mannose-binding lectin is an acute phase inflammatory proteins which act as a soluble receptor that recognizes pathogens. Under normal conditions, MBL can not bind its own network, but in a state of cellular hypoxia, it can induce glycosylated cell surface causes precipitation of MBL followed by activation of complement. Mannose-binding lectin binds to the surface of the pathogen and has a major role in the innate immune response through the ability to increase opsonization pathogens and activating the complement cascade via the lectin pathway [2]. Previous study showed that there was an increase of MBL level in adult chronic RHD patients, which led to the speculation that the MBL caused complement activation contributing to the pathogenesis of RHD [3, 4]. However there has been no study examining the correlation between MBL levels and the severity of valvular regurgitation and heart failure in pediatric RHD.

There is suspected role of oxidative stress in the pathophysiology of rheumatic heart valve disease.

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However, the underlying mechanisms are still not widely known. The balances between oxidant and antioxidant systems are impaired in many inflammatory diseases. Increased oxidative stress decreases immune system function and plays a role in the pathogenesis of autoimmune disorders, due to cell apoptosis. Consequently, the oxidative stress is the result of severe inflammation and expected to contribute to the damage of heart valve disease [5]. Previous research showed that oxidant and antioxidant balance disorders lead to the increase of oxidative stress in acute rheumatic fever [6]. The level of oxidative stress is also increased in patients with severe mitral regurgitation and in the condition of heart failure that can occur as a complication of acute rheumatic fever [7, 8].

Myeloperoxidase (MPO) is a marker of oxidative stress and inflammation. Recent studies showed the role of MPO in increasing the risk of various cardiovascular diseases [9, 10]. Myeloperoxidase is a leukocyte-derived enzyme that catalyzes the formation of ROS and consumes nitric oxide (NO) resulting in endothelial dysfunction [11]. In human, there is an increase of systemic MPO in patients with chronic systolic heart failure [7]. Enzymatically, MPO, together with hydrogen peroxide and chloride, produces hypochlorous acid, an adamant oxidant. Myeloperoxidase is an important contributor to oxygen-dependent tissue damage in autoimmune and chronic inflammatory diseases [12]. It is known that there were chronic inflammation and autoimmune reaction in the case of rheumatic fever [4]. Until now, there was no study that examined the MPO level in pediatric RHD patients and the relationship of MPO level and the severity of valvular regurgitation and heart failure in pediatric RHD patients.

MATERIALS AND METHODS

Design and study population

This case-control study was conducted at Paediatric Cardiology Clinic dr. Saiful Anwar General Hospital (SAGH) Malang, Indonesia from 1st June 2014 to 31st September 2014. This study enrolled 32 children who were divided into two groups, RHD patients, and healthy children as a control. The patient group consisted of 16 RHD patients who came for their regular follow up at Pediatric Cardiology Clinic SAGH, during the period of the study (consecutive sampling). The control group consisted of 16 healthy volunteer children.

The inclusion criteria for patient group were children aged 6 to 14 years old, who were diagnosed with RHD, either with clinical heart failure or not, and had taken therapy for less than 2 years depending on the severity of the disease. Subjects who had acute inflammation, infection, autoimmune diseases, hematologic disorder, and other cardiovascular diseases were excluded from this study.

Definition used in this study

The diagnosis of RHD was determined according to Jones criteria which were modified by the world health organization (WHO) in 2002-2003 for the diagnosis of rheumatic fever (RF) and RHD.13 The valvular abnormalities were identified by echocardiography performed by cardiologist consultant pediatrician, using color jet flow area to determine the severity of valvular regurgitation. The severity of valvular abnormalities were classified into mild, moderate, and severe valvular regurgitation. In this study, the heart failure severity was classified using Ross score classifications when the patient was admitted [14].

Ethical consideration

This study protocol was approved by the Research Ethic Committee of the Faculty of Medicine, Brawijaya University dr. Saiful Anwar Public Hospital No. 400/114/K.3/302/2014. Informed consents were obtained from parents or legal guardians of all children.

Blood collection

Peripheral blood samples were aseptically collected from the patient and control group subjects in the Pathology Clinic Laboratory of dr. Saiful Anwar General Hospital. The MBL from plasma concentration was quantified using sandwich ELISA kit (*Elabscience*). The MPO from plasma concentration was quantified using sandwich ELISA kit (*BioLegend*) according to the instructions of the manufacturer.

Statistical analysis

Data was entered and analyzed using *SPSS statistical package version 21*. The independent t-test was used to analyze the difference of MBL and MPO levels in the patient group compared to control group. One way ANOVA was used to differentiate MBL and MPO levels among mild, moderate, and severe valvular regurgitation and also among mild, moderate, and severe heart failure. For all tests, a p-value of less than 0.05 was significant with 95% confidence intervals.

RESULTS AND DISCUSSION

The 16 children were suffering from RHD and 16

Table 1. Characteristics of the subjects

Characteristic of subject	*Patient (n=16)*	*Control (n=16)*
Sex:		
Male	7 (7/16)	7 (7/16)
Female	9 (9/16)	9 (9/16)
Age (Year)	10.84 ± 2.5	10.44 ± 1.15
Most populated area:		
Yes	11 (11/16)	6 (6/16)
No	5 (5/16)	10 (10/16)
Nutritional status:		
Undernutrition	10 (10/16)*	3 (3/16)
Good	4 (4/16)	10 (10/16)*
Overweight	2 (2/16)	3 (3/16)
Valve regurgitation:		
Mild	6 (6/16)	-
Moderate	5 (5/16)	-
Severe	5 (5/16)	-
Heart Failure:		
Yes	12 (12/16)	-
No	4 (4/16)	-
Duration of treatment:		
< 12 months	8 (8/16)	-
≥12 months	8 (8/16)	-

Note: * In this group of patients showed the majority nutritional status was malnutrition, whereas the control group showed the majority nutritional status was good nutrition.

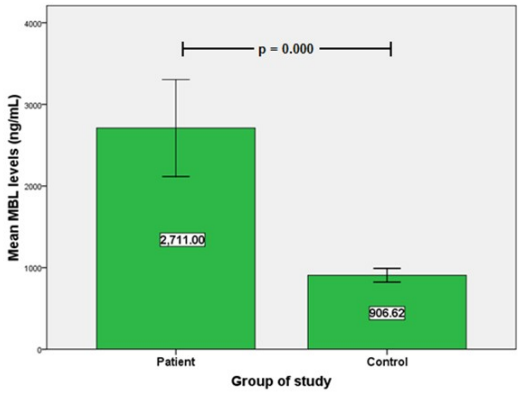

Figure 1. Comparison of MBL levels between patient and control group. The MBL levels of patient group as significantly higher (p = 0.00) than control group

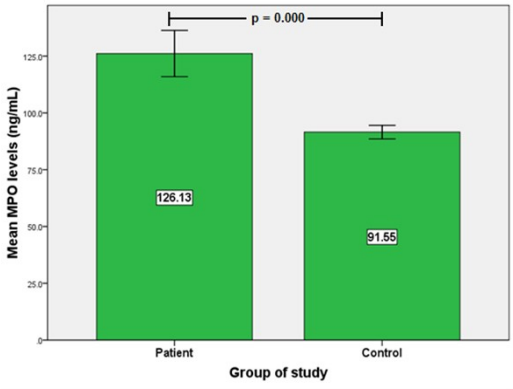

Figure 2. Comparison of MPO levels between patient and control group. The MPO levels of patient group is significantly higher (p = 0.00) than control group

healthy children as a control group. The mean age in RHD patients and control group were 10.84 ± 2.5 years old and 10.44 ± 1.15 years old, respectively. In the patient group, 7 of 16 subjects were male, and 10 subjects were with undernourished status. In control group, 7 of 16 subjects were male, and 10 subjects were with good nutritional status. From the RHD patients, 6 patients had mild valvular regurgitation, 5 patients had moderate valvular regurgitation, and the other 5 had severe valvular regurgitation. There were 12 patients suffered from heart failure. Therapy was administered in various time from 2 months to 2 years to each subject (Table 1).

The mean of plasma MBL level in the RHD patients was 2711 ± 14 ng/mL, higher than the control group with mean MBL level of 906 ± 20 ng/mL (Figure 1). The average of plasma MPO level in RHD patients was 126.13 ± 24 ng/mL, higher than the control group, 91.55 ± 7 ng/mL (Figure 2). Statistical analysis using independent sample t test showed a significant difference in both MBL and MPO levels in RHD patients compared to the control group.

The mean level of MBL and MPO among mild, moderate, and severe valvular regurgitation patients analyzed using one-way ANOVA showed a significant difference. There was a very strong correlation between MBL level and severity of valvular regurgitation (p = 0.00, r = 0.94) and a strong correlation between MPO level and severity of valvular regurgitation (p = 0.00, r = 0.86). The LSD analysis comparing the MBL and MPO levels among mild, moderate, and severe valvular regurgitation showed that the differences occurred in severe valvular regurgitation patients (Figure 3 dan 4).

The test results comparing the mean levels of both MBL and MPO in RHD patients between patients with and without heart failure using independent sample t-test showed no significant difference with p = 0.07 and p = 0.11. The mean level of MBL and MPO among mild, moderate, and severe heart failure patients ana-

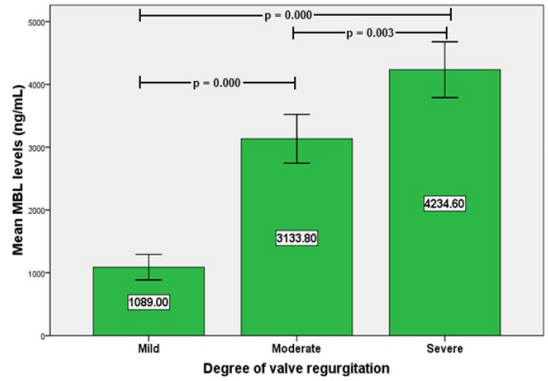

Figure 3. Comparison of MBL levels among mild, moderate, and severe valve regurgitation. The LSD analysis to compare MBL levels between mild to moderate valve regurgitation (p = 0.00), moderate to severe valve regurgitation (p = 0.03), and mild to severe valve regurgitation (p = 0.00)

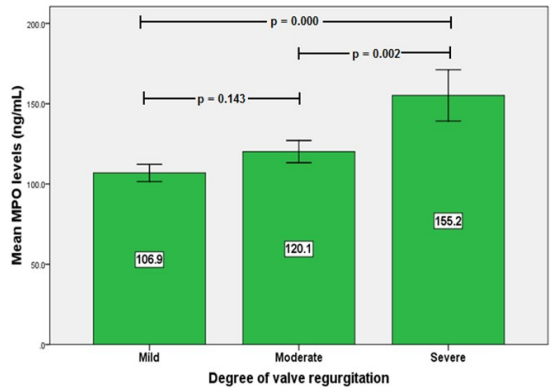

Figure 4. Comparison of MPO levels among mild, moderate, and severe valve regurgitation. The LSD analysis to compare MPO levels between mild to moderate valve regurgitation (p = 0.14), moderate to severe valve regurgitation (p = 0.02), and mild to severe valve regurgitation (p = 0.00)

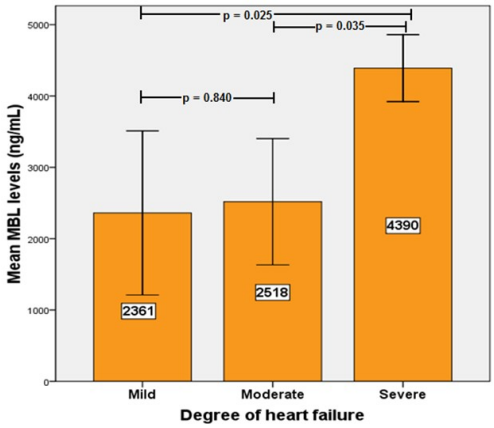

Figure 5. Comparison of MBL levels among mild, moderate, and severe heart failure. The LSD analysis to compare MBL levels between mild to moderate heart failure (p = 0.84), moderate to severe heart failure (p = 0.04), and mild to severe heart failure (p = 0.03)

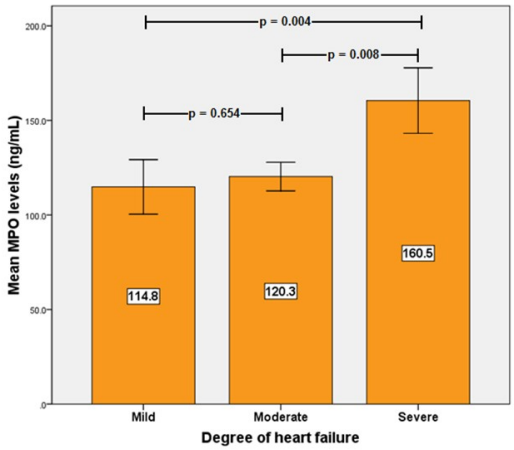

Figure 6. Comparison of MPO levels among mild, moderate, and severe heart failure. The LSD analysis to compare MPO levels between mild to moderate heart failure (p = 0.65), moderate to severe heart failure (p = 0.01), and mild to severe heart failure (p = 0.00).

lyzed using one-way ANOVA showed a significant difference (p = 0.00). The LSD analysis to compare the MBL and MPO levels among mild, moderate, and severe heart failure was indicated in Figure 5 dan Figure 6.

The mean age of children in RHD patients was 10 years and 8 months. The incidence of ARF and RHD is often found in children aged 6-15 years old. Based on epidemiological studies, ARF/RHD are affected by low socioeconomic and environmental factors associated with the densely populated area, poor ventilation, and inadequate nutrition [15]. In this study, the majority of patients had poor nutritional status (10 of 16 children).

The most commonly found valve lesion in RHD is mitral regurgitation. In severe condition, it can lead to heart failure [7]. Study by Kironget et al. (2014) investigating the characteristics of echocardiography in 84 children with RHD showed that most patients had severe valvular abnormalities [16]. In this study, from 16 patients with RHD, 6 patients suffered from mild valvular regurgitation, 5 people suffered from moderate valvular regurgitation, and 5 others suffered from severe valvular regurgitation. The 12 patients suffered from heart failure.

It is known that MBL binds to N-acetyl-β-D-glucosamine tightly in the streptococcal cell wall and sub-

sequently lead to the deposition of complement and opsonization [17]. Mannose-binding lectin concentration in healthy Caucasians is approximately 800–1000 μg/L [3]. This study found that the mean level of MBL was higher in children with RHD compared to healthy children, 1211 ± 14 ng/mL vs 906.6 ± 7 ng/mL. Some previous studies also showed increased levels of MBL in RHD [3, 4, 18].

A study by Schafranski et al. (2004) showed that there was a significant increase of MBL level in adult RHD patients compared to the control group. This study suggested that high level of MBL might cause undesirable complement activation in RHD patients and contributed to the pathogenesis of rheumatic cardiomyopathy. The high level of MBL in chronic RHD patients reinforces the presence of chronic inflammation activity contributing to valvular damage via complement activation in RHD patients [3]. Subsequent studies confirmed that high MBL level (> 2.80 ng/mL) increased the risk of chronic RHD (OR 2.91) compared to healthy control [4].

The high serum MBL level has been associated with persistent inflammation and tissue damage. In addition, MBL can act as immunomodulatory molecules inducing higher secretion of cytokines in the inflammatory process. The complement activation occurs both in acute and chronic cardiac injury.3,18 A prospective cohort study in 182 patients with chronic heart failure showed that complement activation was strongly correlated with chronic heart failure outcome. Especially, the high level of activated C3, C3a could be used as a predictor of heart failure severity, and also associated with hospitalization and mortality [19]. This study found that the increased level of MBL was parallel with the severity of valvular regurgitation and heart failure RHD patients. Chronic inflammatory disease induces endothelial dysfunction, and it is known that heart failure is correlated with the severity of valvular damage in RHD [20].

Myeloperoxidase is an enzyme released by neutrophils which stimulate and catalyzes reactive oxidants, free radicals and nitric oxide formation, which will cause tissue damage. Myeloperoxidase has been widely used as a marker of oxidative stress in various cardiovascular and autoimmune diseases [6, 21]. This study was the first study using MPO biomarker in assessing oxidative status in pediatric RHD patients The results of this study showed that there was a significant increase of MPO level in RHD patients compared to the control group, 126.13 ± 24 ng/mL vs. 7.26±91.55 ng/mL.

Rheumatic fever has the characteristics of an autoimmune disease triggered by streptococcal antigenic cross-reactivity. A study investigating blood plasma of 38 patients aged 3-15 years old with chronic RHD and ARF showed a high level of oxidative stress markers in these patients [22]. There was a study that demonstrated that high oxidant protein products in chronic RHD patients confirmed that there was a chronic inflammatory activity in RHD patients. Increased oxidative stress is expected to play a role in the pathogenesis of autoimmune disease due to its contribution to inflammation and cell apoptosis. As a consequence, the oxidative stress, as a result of severe inflammation, is predicted to have a major role in valvular damage in RHD [6, 23].

A study by Tang et al. (2006) showed that there was a correlation between elevated MPO level and degree of heart failure [8]. Increased MPO is associated with endothelial dysfunction, which is the pathophysiological process in heart failure patients and is often associated with a worse prognosis [24]. Our study found that elevated MPO level was parallel with the degree of valvular regurgitation and heart failure in RHD patients.

Rheumatic valve lesion is a chronic inflammatory process, T cell infiltration to the valves plays a role in the incidence of heart valve regurgitation and stenosis. Mannose-binding lectin has an important role in the inflammatory reaction through the activation of complement. Further, complement activation may induce some inflammatory effects, such as the expression of adhesion molecules, chemotaxis and leukocytes activation, the release of ROS, as well as the secretion of cytokines and chemokines [25]. There are various evidence showing the pro-inflammatory role of MBL in tissue damage and severity of other inflammatory diseases, particularly in cardiovascular disease [4, 5].

The balance between oxidants and antioxidants is impaired in various inflammatory diseases. As a consequence, the oxidative stress generated by the severe inflammation contributes to valve damage [5]. A study by Chen et al. (2012) showed that the increased inflammation was significantly correlated to the increase of oxidative stress. Under stressful conditions such as inflammatory processes mediated by oxidative stress, the cell surface undergo glycosylation and thus can be targeted in MBL/ficolin binding. This in turn will lead to pathological complement activation, and inflammation [26]. *In vitro* studies showed that oxidative stress caused formation of MBL ligand activating complement in the endothelial cells [2].

CONCLUSION

In conclusion, the present work demonstrated a significant increase in MBL and MPO levels in RHD patients. The increases in MBL and MPO levels are correlated with the severity of valvular regurgitation in RHD patients and heart failure, especially in severe condition. This result supports the theory of inflammation and oxidative stress in RHD, providing a new biomarker candidate that can predict the presence of severe valvular regurgitation and worsening of heart failure in RHD.

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