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Difference in the Level of p-Selectin Blood Edge between Uni-Valvular and Multivalvular in Rheumatic Heart Disease

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Abstract. Rheumatic heart disease remains a major medical problem of children and young adults. Rheumatic heart disease is sequelae of acute rheumatic fever, a bacterial infection Group A beta Hemoliticus streptococci (GAS) in the pharynx. It is based on genetic predisposition and an autoimmune process causing defect of heart valves, hemodynamic changes, endothelial damage. Further, it is usually illustrated by elevated levels of P selectin. This study was conducted to prove the difference between peripheral P selectin level in multivalve and univalve (mitral stenosis) in rheumatic heart disease. This cross sectional study involving 37 RHD patients consists of 18 univalve (mitral stenosis), 19 multivalve, and 22 healthy controls. P selectin levels were collected through peripheral vein and echocardiography was performed. Peripheral P selectin was significantly higher in univalve and multivalve rheumatic heart disease than normal. Peripheral P selectin in multivalve group was higher than univalve group (2,384ng/mL vs 2,028ng/mL; $p < 0.05$). There was significant difference of peripheral P selectin between multivalve and univalve (mitral stenosis). P selectin potentially has an important role as an indicator of inflammation and progression of valve damage in mitral stenosis, rheumatic heart disease.

1. Introduction

Rheumatic heart disease (RHD) remains a significant cardiovascular disease in the world. In developed countries, rheumatic heart disease is still a medical and public health problem because it affects children (5-15 years) and more productive young adults with women more than men. Rheumatic heart disease (RHD) is a sequelae of acute rheumatic fever caused by the Hemoliticus Streptococcus (GAS) Group A beta infection in the pharynx region [1,2]. Repeated GAS infection after five weeks will cause progressive and persistent heart valve lesions. Excessive immune response to specific bacterial epitopes is the basis of the pathogenesis of this disease [3].

In developing countries, the prevalence ranges from 7.9 to 12.6 per 1000 school children and is relatively constant [4]. The epidemiological transition - the complex changes in patterns of disease in populations from a dominance of infectious disease to non-communicable disease (NCD)[5] - has had an enormous effect on the burden of disease and nature of healthcare in high income countries (HICs). In low and middle income countries (LMICs) the high burden of infectious disease and NCD coincide, continuing to challenge the management of disease and healthcare resourcing [6]. Globally, infectious disease and NCD epidemics are converging in terms of disease complexity, dual causality and demand on services [7].



Generally, the most common valve lesions in rheumatic heart disease are 90-95% Mitral valve incompetence (MI) and mitral stenosis (MS)[8]. Patients might be diagnosed with rheumatic heart disease after an acute rheumatic fever attack. However, the disease is often diagnosed in patients who were previously asymptomatic or who do not recall acute rheumatic fever symptoms or episodes. Most patients present after the onset of shortness of breath at ages 20–50 years [9]. Clinical diagnosis is based on pathological valvular heart murmur detected during auscultation. Mitral valve incompetence is the most common valvular lesion in patients with rheumatic heart disease, particularly in the early stages [8].

The P-selectin adhesion molecule attracts attention because of its role in modulating the interaction between blood cells and endothelium, and also the possibility of using dissolved forms as plasma predictors of the possibility of severe cardiovascular events. Increased P-selectin expression was found on active atherosclerotic plaques. On the contrary, inactive fibrotic plaques do not show P-selectin expression. Further, animals that do not express P-selectin show a reduced tendency to form atherosclerotic plaques [10]. P-selectin also participates in the process of inflammatory cell interactions with endothelium, which activates platelets. This process is accompanied by thrombosis in cardiovascular diseases, including rheumatic heart disease [11]. P-selectin is significantly increased in patients with moderate to severe mitral stenosis with atrial fibrillation [12]. At present, there is no research that analyzes P selectin levels of peripheral blood in multivalvular. Based on these data, the researcher wanted to analyze the differences in peripheral blood P selectin levels between univalvular and multivalvular in rheumatic heart disease.

2. Methods

This study was an observational analytic study using a cross-sectional design and was carried out in Camellia Room of Dr. Soetomo Hospital Surabaya. The study population was patients with univalvular or multivalvular rheumatic heart disease who went to the Heart Polyclinic or who were hospitalized in the Camellia Room of Dr. Soetomo Hospital, Surabaya. Inclusion criteria are men or women aged 15-56 years, willing to participate in the study and sign an informed consent and having rheumatic heart disease. Additionally, from history, there has been a history of rheumatic fever, medical records, and old echocardiography. However, subjects having undergone Percutaneous Transvenous Mitral Commissurotomy (PTMC) actions, Mitral Valve Replacement (MVR), coronary heart disease, diabetes mellitus, hypertension, obesity, high LDL-C levels, active smokers, elderly, pregnant, and having suffered infective endocarditis or acute rheumatic fever were excluded from the study. Independent variables are univalvular and multivalvular while dependent variable are increase in P selectin. Furthermore, confounding variables are atherosclerosis, autoimmune diseases, allergic diseases and malignancies.

Measurements of P selectin levels were carried out using a quantitative sandwich immunoassay technique examination with units of ng / mL. The minimum value of P selectin detected, used <0.5ng / ml (human soluble parameter P-selectin immunoassay). Mitral stenosis was measured by assessing the narrowing of the mitral valve orifice <4.0cm² caused by complications of acute rheumatic fever. Mitral stenosis was also marked by mean gradient 4mmHg with at least two morphologies of mitral stenosis, namely, mitral valve thickening, presence of mitral valve commissure fusion, mitral chordate shortening and fusion. Calcification and restriction of mitral valve movements, especially the mitral posterior valve, were measured and seen through echocardiographic examination in parasternal long axis projection, apical four chamber, apical two chamber according to World Heart Federation (WHF) criteria. Aortic regurgitation characterized by the presence of jet regurgitation length \geq 1cm measured from the vena contracta, can be seen in two different viewpoints, a picture of mosaic color jet with peak velocity \geq 3m/s, regurgitant jet seen in the systole (mitral valve) and diastole phase (aortic valve), which is measured and seen through an echocardiographic examination according to WHF criteria. The examination is carried out using an echocardiography device called Vivid 7 GE. Data obtained from the examination results were analyzed using Kolmogorov-Smirnov test using SPSS 20.0.

3. Results

3.1. Characteristics of subjects

Patients with rheumatic heart disease were dominated by women at 78.38% with a mean age of 41.05 ± 9.31 years. Most of the patient's data were obtained from cardiac outpatient at 81.08%, with a mean length of complaints of 4.00 ± 2.01 years. Most patients experienced rhythm of atrial fibrillation of 81.08% and cardiomegaly with a mean CTR of $60.84 \pm 4.63\%$. Most patients have controlled complaints with NYHA FC II (91.89%).

Table 1 . Characteristics of patients with rheumatic heart disease (n = 37)

Variables	n(%) or mean \pm SB
Age (years)	41.05 ± 9.31
Gender	
Male	8 (22.62)
Female	29 (78.38)
Care unit	
Outpatient clinic	30 (81.08)
Low care unit	7 (18.91)
Duration of complaint (years)	4.00 ± 2.01
Body mass Index (Kg/m ²)	21.06 ± 3.25
Blood Pressure	
Systolic (mmHg)	102.16 ± 11.58
Diastolic (mmHg)	66.76 ± 7.09
ECG	
Sinus	7 (18.91)
Atrial Fibrillation	30 (81.08)
NYHA FC II	34 (91.89)
NYHA FC III	3 (8.11)
CTR	60.84 ± 4.63

ECG: Electrocardiography

NYHA: New York Heart Association

FC: Functional Class

CTR: Cardio Thoracic Ratio

3.2. Echocardiogram characteristics of patients with mitral stenosis in rheumatic heart disease.

Patients with mitral stenosis RHD generally have severe stenosis with mean MVA by planimetry of 0.77 ± 0.26 cm², mean MVA by PHT of 0.74 ± 0.24 cm² and Mitral Valve mean Pressure Gradient (MV mean PG) of 12.68 ± 4.63 mmHg. Patients have a good hemodynamic status, which is characterized by mean Estimated Right Atrial Pressure (Est. RAP) of 10.27 ± 3.53 mmHg. The following are the characteristics of echocardiogram of RHD patients listed in Table 2 and 3.

Table 2. Characteristics of echocardiogram of mitral stenosis (n=18)

Variables	n (%) or mean \pm SB
MVA ^a by Planimetry (cm ²)	0.77 ± 0.26
MVA by PHT (cm ²)	0.74 ± 0.24
MV mean PG ^b (mmHg)	12.68 ± 4.63
Est RAP ^d (mmHg), mean \pm SD	10.27 ± 3.53
Pulmonary Hypertension	
Mild	4 (29.73)

Moderate	6 (32.43)
Severe	8 (37.84)
Est PASP	75.17 ± 22.80
LA Thrombus	
No Thrombus	15 (91.89)
Thrombus	3 (8.11)
LASEC	
No LASEC	11 (54.05)
LASEC	7 (45.95)
LA Diameter (cm)	4.85±1.23
EF ^s by Teich (%)	60.69±5.59
EF by Biplane (%)	60.25±4.78

MVA: Mitral Valve Area
MV mean PG: Mitral Valve Mean Pressure Gradient
Est RAP: Estimated Right Atrial Pressure
Est PASP: Estimated Pulmonary Artery Systolic Pressure
LA: Left Atrium
LASEC: Left Atrial Spontaneous Echo Contrast
EF: Ejection Fraction

Table 3 . Echocardiogram characteristics of multivalvular patients (n = 19)

Variables	n (%)
Pure MS	8 (42.11)
MS + MR	11(5.89)
AS	5 (26.32)
AR	
Mild	10 (52.63)
Moderate	9 (47.36)

MS: Mitral Stenosis
MR: Mitral Regurgitation
AS: Aortic Stenosis
AR: Aortic Regurgitation

3.3. Characteristics of patients with pure mitral stenosis and multivalvular rheumatic heart disease

The average age of patients with pure RHD stenosis was 39.44 ± 9.25 years and the mean age of multivalvular patients with RHD was 42.58 ± 9.35 years. Physical examination parameters between patients with pure mitral stenosis and multi valvular rheumatic heart disease include systolic and diastolic blood pressure and body mass index. Echocardiogram parameters between patients with pure mitral stenosis and multi valvular RHD include MVA plannimetry, IPM MVA, mean MVA MV, PASP estimation and LA diameter. Table 4 shows the characteristics of pure and multivalvular mitral stenosis RHD.

Table 4. Characteristics of mitral stenosis and multivalvular rheumatic heart disease

Variables	Pure univalvular (Mitral stenosis) (n=18)		Multivalvular (n=19)		P
	Mean±SB	Median (Min-Max)	Mean±SB	Median (Min-Max)	
Age (years)	39.44±9.25	41.50 (23-58)	42.58±9.35	42 (22-59)	0.313*
Systolic blood pressure (mmHg)	104.44±12.47	100 (90-130)	100±10.54	100 (80-120)	0.249*

Diastolic blood pressure (mmHg)	68.33±6,18	70(60-80)	65.26±7,72	70(50-80)	0.245**
BMI (kg/m ²)	21.49±3.39	20.95(14-27)	20.67±3.16	22.03 (13-25)	0.451*
Est PASP (mmHg)	78.19±20.88	70.61 (45-118)	72.20±24.68	73.01 (35-116)	0.432*
MVA ^c plannimetry (cm ²)	0.74±0.22	0.70 (0.4-1.2)	0.80±0.29	0.80 (0.3-1.4)	0.440*
MVA PHT ^d (cm ²)	0.73±0,20	0.71(0.4-1.14)	0.74±0.28	0.66 (0.3-1.3)	0.919*
MVA mean PG ^e (mmHg)	13.16±5.53	11.92(4-26)	12.22±3.69	11.69 (6-23)	0.549*
LA ^f diameter (cm)	4.51±0.55	4.55 (3.3-5.7)	5.17±1.59	4.7 (2.2-8,,8)	0.105*

BMI: Body Mass Index

PASP: Pulmonary Arterial Systolic Pressure

MVA: Mitral Valve Area

PHT: Pressure Half Time

PG: Pressure Gradient

LA: Left Atrium

4. Discussion

This research was conducted by trying to control several factors potentially increasing selectin P levels, such as coronary heart disease, diabetes mellitus, hypertension, obesity, allergies and autoimmune diseases, high LDL levels, smoking, class IV NYHA heart failure, acute infection / sepsis and malignancy . This study also controls several factors that can reduce P selectin levels of peripheral blood, such as PTMC and MVR. This is done so that the analysis of P selectin levels is really only related to rheumatic heart disease. This study showed that most patients with univalvular rheumatic heart disease were women (78.38%). The data are in accordance with several studies of rheumatic heart disease that show more women are affected by RHD than men [13].

There are several things that explain the occurrence of homogeneity in severity of severe mitral valve stenosis. First, the place of research was conducted at Dr. Soetomo General Hospital, which is a type A hospital and as a referral center in Eastern Indonesia. The types of cases managed are cases of terminals that have not been handled in primary or secondary healthcare facilities in their respective regions. Second, the lack of proper medical treatment carried out by primary and secondary healthcare facilities before. This can be due to the lack of health workers, lack of knowledge and skills of health workers and the lack of facilities and infrastructure to support health. Handling of rheumatic heart disease that requires intervention and surgery strategies is also an obstacle to primary and secondary healthcare facilities so it must be referred to tertiary health services, in this case RSUD dr. Soetomo. Third, the delay in management, including the timing of the patient in realizing th illness and checking into a health worker. The limitation of health facilities and infrastructure is one of the factors that still causes extensive rheumatic heart disease in developing countries in addition to poor nutrition and unhealthy dense home conditions [2].

Selectin is a carbohydrate binding molecule that is bound to fucosylated and sialylated glycoprotein ligands, and is found in endothelial cells, leukocytes and platelets. Selectin is involved in the transfer of natural immune system cells, T lymphocytes and platelets (Sperandin). P-selectin attracts the most attention because it is expressed by platelets and endothelial cells in certain conditions. P-selectin (CD62P), which is the largest selectin, with mass 140kDa, stretches approximately 40nm from the endothelial surface [14]. P-selectin levels dissolved in venous plasma MS patients were significantly higher than healthy individuals or patients with atrial fibrillation. In patients with MS, dissolved P-selectin concentrations in the left atrial plasma showed no significant differences with the right atrium, femoral vein, and arteries. This is in line with femoral studies in Chen in 2004 where there were 20 patients with mitral stenosis, a significant increase in P selectin in patients with mitral stenosis compared with controls and no difference in P selectin in femoral artery venous blood or in the right and left atrial [15]. The mechanism that plays a role is thought to be related to platelet activation and endothelial dysfunction, which persists along with the disease. Previous studies showed that, despite

significant decrease in mitral valve area, mean pulmonary and atrial artery pressure after PTMV, the decrease in P-selectin plasma was not significant [16]. In this study, peripheral blood P selectin levels were found to be significantly increased in rheumatic heart disease compared to normal subjects (0.033ng/mL vs. 0.003ng/mL; $\rho < 0.05$). There was also a significant difference between univalvular rheumatic heart disease and normal subjects (0.026ng/mL vs. 0.022ng/mL; $\rho < 0.05$). In addition, significant difference was found between multivalvular rheumatic heart disease and normal subjects (0.020ng/mL vs. 0.013ng/mL; $\rho < 0.05$), which is consistent with Tarnow (2005) where there was an increase in P selectin levels in multivalvular dogs compared to controls [17]. Tarnow also compared P selectin levels of peripheral blood between patients who experienced atrial fibrillation and sinus rhythm and found a difference (0.017 vs 0.001ng/ml; $\rho < 0.05$) [17]. Another study obtained significant differences in P selectin levels in patients with mitral stenosis with atrial fibrillation groups and normal subjects [18]. Marten et al. showed a significant decrease in P selectin levels after PTMC action at weeks 2 and 4, 2-3 months post MVR, presumably a decrease in P selectin is associated with platelet activity in hemodynamic disorders of rheumatic heart disease [18,19]. From the various findings above, the researcher believes that examination of peripheral blood P-selectin levels has the potential to play an important role as an indicator of inflammation and progression of valve damage that occurs in patients with mitral stenosis, rheumatic heart disease. Examination of P selectin levels of peripheral blood rheumatic heart disease can also be useful as a therapeutic evaluation parameter.

5. Conclusion

In conclusion, P selectin levels of peripheral blood are higher in multivalvular than univalvular rheumatic heart disease. Additionally, P selectin levels of peripheral blood are higher in univalvular rheumatic heart disease than normal and P selectin levels of peripheral blood are higher in multivalvular rheumatic heart disease than normal.

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