



Role of Carboxy Terminal Propeptide of Type I and Type III Procollagen (PICP and PIIICP) Toward the Severity Degree of Mitral Valve Regurgitation in Children's Rheumatic Heart Disease

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Abstract

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BACKGROUND: Rheumatic heart disease (RHD) is recognized as a heart disease that occurs as a result of sequelae in acute rheumatic fever (ARF), characterized by the occurrence of defects in the heart valves. The most common manifestation of childhood RHD is mitral regurgitation (MR). The role of inflammation and oxidative stress in RHD also involves several components consisting of carboxy-terminal pro-peptide of Type I procollagen (PICP) and carboxy-terminal pro-peptide of Type III procollagen (PIIICP).

AIM: The aim of this study was to know whether PICP and PIIICP can be used to measure the severity level of mitral valve regurgitation.

METHODS: This research is considered as descriptive-analytic research, and using cross-sectional analysis. Forty RHD patients underwent echocardiographic examinations to measure Wilkin and effective regurgitant orifice area scores. Patients were classified into ARF without valve abnormalities, mild, moderate, and severe MR. PICP and PIIICP were with ARF through venous blood and ELISA was examined. Data were analyzed by employing SPSS 22 with $p = 0.05$. Wilkins scores and PICP levels have a regression coefficient of 0.296 with a p -value of 0.032.

RESULTS: There was a significant difference in PICP level among the studied sample groups with a $p = 0.012$, ($p < 0.05$), with insignificant difference in PIIICP level among sample groups with a $p = 0.083$, greater than $\alpha = 0.05$ ($p > 0.05$). Wilkins scores and PICP level have a regression coefficient of 0.296 with a $p = 0.032$ ($p < 0.05$), while PIIICP level has a regression coefficient of 0.093 with a $p = 0.568$ ($p > 0.05$).

CONCLUSION: There is no significant increase indicated on PIIICP level, but PICP level indicates a significant increase in RHD group with severe mitral valve abnormalities. PICP can be used to measure the severity level of mitral valve regurgitation.

Introduction

Rheumatic heart disease (RHD) becomes a major cause of heart valve disease worldwide and is a leading cause of cardiovascular death in children at developing countries [1]. According to the World Health Association Report [2], the annual incidence of acute rheumatic fever (ARF) in children (age 5–14 years) reaches to 300,000–350,000. In addition, there are around 1.5% mortality rates of RHD sufferers with carditis annually [2]. The mitral valve was the most commonly affected part of the heart valve (± 65 –70%), followed by the aortic valve ($\pm 25\%$), while the tricuspid and pulmonary valves were rarely affected. The most common valve abnormality in children concerns with mitral valve regurgitation mitral regurgitation (MR) [3].

Group A beta-hemolytic streptococcus (GABHS) polysaccharides and cardiac valve glycoprotein have similarities that are both considered

as N-acetyl glucosamine. This structural similarity (molecular mimicry) causes a cross-reaction between GABHS and heart valve tissue [4]. Heart valve tissue that was not originally an antigen (non-self-antigen) turned into an antigen (self-antigen), thereby triggering a response to humoral immunity [5] and cellular immunity which in turn triggers inflammation [6].

Inflammation and oxidative stress in RHD involve several components, including carboxy-terminal propeptide of Type I procollagen (PICP) and carboxy-terminal propeptide of Type III procollagen (PIIICP) [7]. Increased serum of PICP and PIIICP has been found in various heart diseases such as cardiomyopathy, hypertensive heart disease, adult heart failure, and adult rheumatic mitral valve disease [8], [9], [10]. However, relevant studies were unfortunately limited to examining the levels of PICP, PIIICP in pediatric RHD patients, and the relationship between PICP and PIIICP levels to the severity of mitral valve regurgitation in pediatric RHD.

Echocardiography serves as the gold standard to determine the severity of valve involvement in RHD. Nevertheless, such examination has been widely unavailable; thus, an alternative examination is proposed as a predictor of valve damage degree in patients with RHD. One developed method is the examination of biomarker remodeling of P1CP and PIIICP [11].

Based on this background, the researchers initiated to examine the relationship between increased carboxy-terminal propeptide of Type I and Type III procollagen (PICP and PIIICP) to the severity of mitral valve regurgitation in pediatric RHD patients.

Materials and Methods

This research is considered an observational analytic study with a cross-sectional method. In this study, there were four groups of cases, including pediatric RHD patients with mild, moderate, and severe mitral valve regurgitation and one group of patients with rheumatic fever who did not experience valve abnormalities. This research was conducted at Children Cardiology Clinic at General Hospital Dr. Saiful Anwar Malang and Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya. This research was conducted on July 2019 until fulfilling the number of collected samples in December 2019 with ethical clearance number 400/136/K.3/302/2018.

Sample collection

The study sample involved pediatric patients (aged between 5 and 17 years) who were diagnosed with RHD and underwent outpatient care at Children Cardiology Polyclinic. All study subjects had agreed and signed the research informed consent.

Sampling in this study employs a purposive sampling method with exclusion criteria for patients experiencing inflammation or other acute infections other than ARF or RHD. This research used formula Dahlan, 2010 [12].

$$n = \left(\frac{Z\alpha + Z\beta}{0.5 \ln((1+r)/(1-r))} \right)^2 + 3$$

Sampling of peripheral blood was performed in the Clinical Pathology Laboratory (Central Laboratory) of RSSA by laboratory personnel. The blood volume administered in each study sample was 3 cc for ELISA examination. Blood was inserted into the EDTA vacutainer tube, stored in a cool box, and kept at a temperature of 4°C [13].

Echocardiography

Based on echocardiographic results, forty

patients were diagnosed with RHD based on the 2012 Echo World Heart Federation criteria by determining the severity of mitral valve regurgitation. Morphological mitral valve features are well-visualized on standard 2D echocardiography. This research used Vivid-q 2D echocardiography [14].

The severity of mitral valve regurgitation is determined by transthoracic echocardiography. The severity level is divided into three which are: mild, moderate, and severe by utilizing quantitative and semi-quantitative methods based on the European Association of Echocardiography (2010) recommendations to assess valvular regurgitation (mitral and tricuspid regurgitation), by considering the value of effective regurgitant orifice area, volume regurgitant, and venous contracta. Inflammation which occurs in the mitral valve is echocardiographically assessed by administering the Wilkins Score.

The morphology of the mitral valve was assessed as described by Wilkins *et al.*, based on a semiquantitative grading of mitral valve leaflet mobility, thickening, calcification, and subvalvular thickening, each on a scale of 0–4, with higher scores representing the more abnormal structure. The total echocardiographic score was obtained by adding the scores of each of these individual components. According to this system, a score of 0 would be a totally normal valve, whereas a score of 16 would represent an immobile valve with fibrosis involving the entire leaflet and subvalvular apparatus and severe superimposed calcification [15], [16].

Biochemical marker examination

The technique to measure PICP and PIIICP levels in this study is ELISA with a sandwich technique. PICP level was measured by ELISA paint kit of SEA570Hu, while PIIICP level was measured by paint kit of CEA963Hu [13].

Statistical analysis

The collected data were analyzed by employing SPSS 22 software and multiple linear regression analysis to determine the effect of PICP and PIIICP levels on the severity of mitral valve regurgitation in pediatric RHD patients.

Results

The characteristics of the study involving 40 research samples are presented in Tables 1 and 2 by age, height, weight, total Wilkin Scores, and sex, respectively. Based on Table 1, the data indicate that

the lowest mean age was found in the severe group of 9.7 ± 2.2 years and the oldest average age was found in the moderate group which was 12.7 ± 2.3 . The researcher performed ANOVA indicating $p = 0.148$ ($p > 0.05$), marking that there were no differences in age characteristics between groups.

Table 1: Characteristics of the study sample by age, height, weight, and total Wilkin score

Group	Characteristic			
	Age	Height	Weight	Wilkin score
Normal	10.2 ± 2.2	135.1 ± 13.4	32.7 ± 15.4	0 ± 0
Mild	11.4 ± 3.9	140.2 ± 15	38.2 ± 11.4	0 ± 0
Moderate	12.7 ± 2.3	140.2 ± 15.1	37.3 ± 21.4	1.6 ± 1.1
Severe	9.7 ± 2.2	140 ± 6.6	31.9 ± 6.1	2.7 ± 1.5
p-value	0.148	0.799	0.363	0.000

Likewise, the characteristics of height and weight obtained p-value of more than 0.05 ($p > 0.05$). The results of the test proved that there were no significant differences in the characteristics of age, height, and weight between the studied sample groups.

Table 2: Characteristics of study samples by sex

Group	Sex		p-value
	Male (%)	Female (%)	
Normal	5 (55.6)	4 (44.4)	0.175
Mild	5 (38.5)	8 (61.5)	
Moderate	5 (55.6)	4 (44.4)	
Severe	1 (11.1)	8 (88.9)	
Total	16 (40)	24 (60)	

Table 2 shows that, based on the characteristics of total Wilkin score, $p < 0.05$ which indicates the difference in characteristics of total Wilkin score. Both normal and mild groups have the lowest total score. The highest total Wilkin score was found in the severe group.

Table 2 shows the characteristics of study samples by sex which clearly indicates that the majority of the study samples are female. The result of Chi-square test demonstrating $p = 0.175$ ($p > 0.05$) proves that there are no differences in sex characteristics between groups.

When compared among the mild, moderate, and severe mitral valve regurgitation in children with RHD, the data indicate that the highest average of PICP levels is found in the severe group, which are significantly different from the mild and moderate groups.

Based on the ANOVA test, Figure 1 shows that the $p = 0.012$, ($p < 0.05$). Thus, the researcher concludes that there are significant differences in PICP levels among the studied sample groups.

Figure 2 shows that analysis using the ANOVA test indicates $p = 0.083$, which is greater than $p = 0.05$ ($p > 0.05$). Therefore, it is concluded that there is no significant difference in PIIICP level among the studied sample groups.

Average PIIICP levels increase in the group of children with RHD, in mild, moderate, and severe group. However, increasing PIIICP levels do not occur significantly.

PICP level indicates a regression coefficient of 0.034 with a $p = 0.007$ ($p < 0.05$), concluded that PICP level has a significant effect on the variable of valve

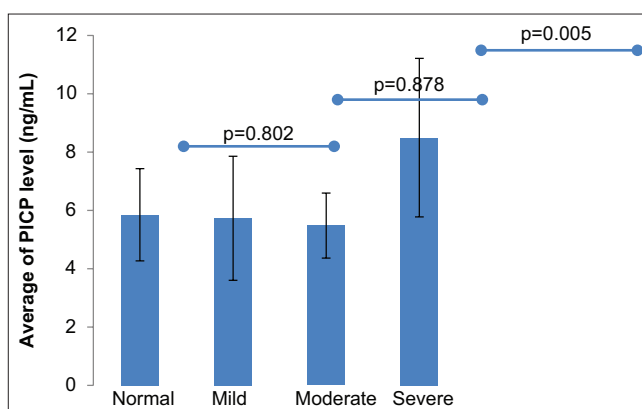


Figure 1: PICP levels of Mitral Valve Regurgitation in Children's RHD

regurgitation in children with RHD. Meanwhile, the level of PIIICP has a regression coefficient of 0.0–0.008 with a $p = 0.303$ ($p > 0.05$).

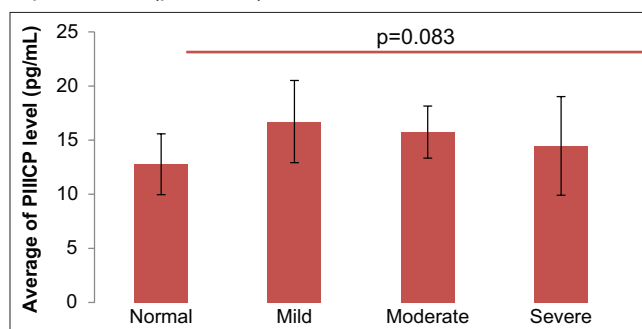


Figure 2: PIIICP level of Mitral Valve Regurgitation in Children's RHD

Wilkins score and PICP level have a regression coefficient of 0.296 with a $p = 0.032$ ($p < 0.05$), concluded that PICP level has a significant effect on Wilkins scores in children with RHD. Meanwhile, PIIICP level has a regression coefficient of 0.093, with a p-value of 0.568 ($p > 0.05$).

Discussion

The sex distribution in this study demonstrates the prevalence of RHD cases which is higher in females than in males but does not show a significant difference. The proportion of RHD based on sex is slightly different from the proportion based on registry data in Australia (1997–2010), indicating approximately 65.8–71.1% of female. Although there are differences in numbers, similarity has been evident; emphasizing that female sex is higher than male.

The results of the study postulate that there are no significant differences in the characteristics of age, height, and weight among the studied sample groups. Thus, such finding indicates that the research sample is homogeneous among groups.

Cardiac extracellular matrix (ECM) consists of collagen Type I (85%) and Type III (11%). Type I and

III collagen are synthesized by cardiac fibroblasts. Cardiac fibroblasts are collagen-producing cells located in the human heart inhibited by mRNA cells for the formation of collagen I and III. Fibrillar collagen is synthesized as procollagen, further broken down by specific proteinases into carboxy (C) – and amino (N) – terminal propeptides. N-terminal Type I or Type III collagen propeptides (PINP and PIIINP) and C-terminal propeptides (PICP and PIIICP) are utilized as a Type I or III collagen synthesis marker. After splitting into propeptides, the triple helix chain will form fibers. During the inflammatory process in RHD, the released cytokines cause differentiation of valvular interstitial cells (VICs) to activate myofibroblasts. VICs are regarded as the most cells in valves producing ECM proteins, including collagen [10].

The role of carboxy-terminal propeptide of Type I (PICP) is released into the bloodstream during Type I collagen synthesis. Therefore, PICP is considered as a marker of Type I collagen synthesis. Likewise, PIIICP is released into the circulation during the synthesis of Type II collagen, which is 85% higher than PIIICP, causing PICP to be more significant than PIIICP.

A significant relationship to PICP in this study is similar to previous studies [10]. In the study, plasma carboxy-terminal propeptide of Type I (PICP) was indicated to increase concentration in MS and MR patients compared to the control groups. PICP levels are strongly related to the mitral valve area in MS. Whereas in MR, these parameters are related to left ventricular diastolic pressure and systolic diameter [10]. PICP level has a positive influence on the severity of valve regurgitation in children with RHD ($p = 0.007$, $p < 0.05$). This finding indicates that PICP level is beneficial to predict the severity of valve regurgitation.

The prior study emphasized that the ratio of Type I/III collagen in the mitral valve and aortic valve in 10 patients with RHD, significantly increased ($p = 0.001$). The amount of collagen was higher in the mitral valve than in the aortic valve ($p = 0.01$), where the ratio of Type I/III collagen was lower in the normal mitral valve compared with the aortic valve ($p = 0.001$). Increased collagen in mitral and aortic valves for patients with RHD was mainly due to an increase in Type 1 collagen [17].

Type 1 and Type 3 collagen are found in cardiac tissue. Although Type 1 collagen is predominant in myocardium, Type 3 collagen is more specific to heart tissue [10], causing PIIICP to have no significant relationship to the degree of heart damage.

In addition, carboxy-terminal propeptides of Type III collagen (PIIICP) are not completely broken down during the conversion of pro-Type III collagen to Type III collagen, by considering the fact that it requires longer steps, removed during fiber degradation. Consequently, the ratio among the released number of Type III collagen, PIIICP, and PIIINP molecules is equivalent [18], causing an insignificant level of PIIICP.

The results indicate a significant difference in the total Wilkin scores among sample groups. Wilkins score includes four following parameters: Mitral valve thickening, valve mobility, valve calcification, and subvalvular thickening. Inflammatory parameters are positively related to biomarkers in the ECM [19], [20]. In this study, PICP and PIIICP levels escalate according to Wilkins score calculations.

Research about Wilkin score and mitral stenosis (MS) showed that in patients with MS, especially asymptomatic patients, the conjunction of the biomarkers score (BNP, tenascin-c, copeptin, and hs-CRP) and Wilkin's score provided higher prognostic value [21].

Conclusion

There is no significant increase on PIIICP level; on the other hand, PICP level indicates a significant increase in RHD group with severe mitral valve abnormalities. PICP level also has a significant effect on Wilkins scores in children with RHD, highlighting that PICP level is advantageous to predict the severity level of valve regurgitation.

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