Heart Sci J 2022; 3(4): 9-15

Contents list available at www.heartscience.ub.ac.id

Heart Science Journal

Journal Homepage : www.heartscience.ub.ac.id

Original Article

Correlation of Urine Albumin Creatinine Ratio and C-Reactive Protein Levels on Carotid Artery Intima-Media Thickness and Flow-Mediated Dilatation Response in Children and Adolescent with Type 1 Diabetes Mellitus at Dr Saiful Anwar Hospital Malang

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ARTICLE INFO

Keywords:
Albumin to Creatinine Ratio;
High Sensitivity CRP;
Carotid Artery Intima-Media Thickness;
Flow-Mediated Dilatation;
Type 1 Diabetes.

ABSTRACT

Background: Early and accelerated atherosclerosis is a major cause of cardiovascular disease and often causes premature death in T1DM patients. In T1DM, atherosclerosis can be detected since adolescence. The initial association between urinary albumin to creatinine ratio (ACR) and c-reactive protein (hs-CRP) with subclinical cardiovascular disease in children and adolescents with T1DM supported findings from previous studies. Imaging tests using ultrasound can detect subclinical atherosclerosis in this patient population. Carotid artery intima-media thickness (cIMT) and flow-mediated dilatation response (FMD) have been frequently used to detect subclinical atherosclerosis.

Objectives: To find correlation between ACR and hsCRP on the thickness values of cIMT and FMD in children and adolescent T1DM patients at Dr Saiful Anwar Hospital Malang

Methods: This is a cross-sectional study with 82 subjects of T1DM patients who routinely control the pediatric outpatient clinic of RSUD Dr. Saiful Anwar Malang, with the research period January – July 2019 and December 2021 – March 2022. Subjects were undergone valcuras ultrasound examination for measurements of cIMT and FMD. Blood sample from subjects were also collected to detect level of ACR and hsCRP. Associations between the study variables were estimated by calculating the Pearson's rank correlation.

Result: There was correlation between ACR with FMD and cIMT (r=-0.593; p=0.000 and r=0.339; p=0.002, respectively). ACR was negatively correlated with FMD and positively correlated with cIMT. There was also correlation between hsCRP with FMD and cIMT (p=-0.375; p=0.001 and r= 0.414; p=0.023, respectively). hsCRP was negatively correlated with FMD and positively correlated with cIMT.

Conclusion: ACR and hsCRP have a correlation with increasing CIMT values and decreasing FMD values in children and adolescents with T1DM patients. Preadolescent children with T1DM displayed evidence of increased low-intensity vascular inflammation, increased cIMT and attenuated FMD measurements. These data suggest that endothelial dysfunction and systemic inflammation are present even in preadolescent children with T1DM

1. Introduction

Atherosclerosis in T1DM can be detected since adolescence. The first important stage towards the development of atherosclerosis is endothelial dysfunction. Microalbuminuria and proteinuria are preceded by an early increase in urinary albumin excretion. The early increase in urinary albumin excretion during adolescence is critical, not only for identifying the risk of developing microalbuminuria and diabetic nephropathy, but may also signal an increased risk of cardiovascular disease to the clinician. The initial association between urinary albumin to creatinine ratio and subclinical cardiovascular disease in adolescents with T1DM supports findings concluded from a cohort study in Australia.[1]

Many epidemiological and clinical studies have revealed that circulating inflammatory biomarkers may influence the likelihood of future cardiovascular events since the inflammatory process involved in the development of atherosclerosis was first postulated. High sensitivity C-Reactive Protein (hsCRP) has been demonstrated to be one of the most significant and useful clinically relevant markers for increased cardiovascular risk among the acute phase proteins examined so far.[2] The study by Babar et al. supports previous clinical research by showing that oxidative stress and atherosclerosis in T1DM begin in childhood.[3]

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https://doi.org/10.21776/ub.hsj.2022.003.04.3
Received 9 July 2022; Received in revised form 30 August 2022; Accepted 15 September 2022
Available online 1 October 2022

Imaging tests can detect subclinical atherosclerosis in patient populations at risk for cardiovascular complications, such as T1DM patients. This test can be used as a reference for primary prevention of cardiovascular complications and reclassification of risk categories, especially in patients with low or moderate risk of cardiovascular complications in the future. Carotid artery intima-media thickness and flow-mediated dilatation response have been frequently used to detect subclinical atherosclerosis.[4]

The purpose of this cross-sectional study was to assess the correlation of urine albumin creatinin ratio (ACR) and high sensitivity CRP (hsCRP) on Carotid Intima-Media Thickness (cIMT) and Flow Mediated Dilation (FMD) values in children and adolescents with T1DM at Saiful Anwar Hospital Malang.

2. Subject and Methods

The Research Ethical Committee of the Faculty of Medicine at Universitas Brawijaya (No. 400/039/K.3/302/2022) approved the procedures utilized in this study. From January to July 2019 and December 2021 to March 2022, 82 research participants were enrolled using the consecutive sampling method, with each participant who met the inclusion criteria being enrolled in the study. All individuals were tested with a complete blood count, ACR, hsCRP, lipid profile, renal and liver function tests, and clinically, followed by cIMT and FMD evaluations. This study was carried out at the outpatient clinic of the Paediatric Endocrinology and Cardiovascular Department at the Saiful Anwar Hospital in Malang, Indonesia.

The T1DM group's inclusion criteria were as follows: has been diagnosed with T1D, is between the ages of 3 and 18, with positif GAD65 immunoassay, and his or her parents/guardians consented to the child's involvement in this study after being informed (informed consent). Exclusion criteria included local and systemic infections, liver dysfunction, impaired renal function, malignancy, and anemia, as well as a three-week history of Vitamin D supplementation, also history taking antihypertensive, anti-inflammatory, or cholesterol-lowering drugs. Using an indirect enzyme-linked immune-absorbent assay (ELISA), the GAD65 assay was used to validate the presence of T1DM in participant.

2.1 Measurement of ACR

Albumin can be found in the urine of patients with early-stage renal impairment. The method used in this study to evaluate albuminuria was to measure urine ACR in spot urine samples. ACR is calculated by dividing the albumin concentration in milligrams by the creatinine concentration in grams.

2.2 Measurement of hsCRP

hsCRP test is more sensitive than the standard CRP test. The high-sensitivity test can detect a slight increase in the normal range of standard CRP levels. Examination using blood serum, then processed by the method of the immunoturbidimetric system.

2.3 Measurement of FMD

Measurement of FMD response of the brachial artery using a Phillips Affinity echocardiography machine with a high resolution L12-5 linear transducer probe. The forearm is equipped with a blood pressure cuff, 5 to 10 cm below the elbow, the brachial artery is scanned longitudinally. The transducer was positioned in the same position to ensure image consistency after the clearest B-mode image of the anterior and posterior intima interfaces between the lumen and the vessel wall was obtained. To optimize the arterial lumen wall interface image, the depth and gain settings were adjusted. The cuff blood pressure was raised to 50 mm Hg above the systolic pressure and maintained for 5 minutes after obtaining a baseline longitudinal image for 30 seconds before the cuff pressure was raised. Longitudinal images of the arteries were recorded continuously for up to 3 minutes after the cuff was deflated. The direct brachial artery diameter change was expressed as a percentage change compared to the vessel diameter before cuff inflation. The percentage change in peak vessel diameter from baseline was used to calculate FMD. FMD percentage is obtained from [(peak diameter - baseline diameter)/baseline diameter] x 100%.[5]

2.4 Measurement of cIMT

European Association of Pediatric Cardiology, American Heart Association, and American Society of Echocardiography Carotid Intima-Media Thickness Task Force all have guidelines for measuring intima-media thickness.[6] The carotid arteries are imaged in a cardiovascular ultrasound facility. After the research partisipants rested for 10 minutes, measurements were taken. The examination is performed with the patient lying comfortably in a supine position. The patient's neck is slightly stretched, and the head is rotated 45 degrees to the opposite side of the side being examined. The carotid arteries were imaged using a high-frequency 12-MHz linear-array transducer (Philips Affinity, L12-5 linear transducer).

To ensure optimal imaging of the vessel wall, ensure that the vessel is as perpendicular to the ultrasound plane as possible. The common carotid artery, carotid bulb, and internal carotid artery in the far wall were all measured when the carotid artery was examined on its longitudinal axis using various scanning angles (anterior and lateral). The focus should be around 30–40 mm, the frame rate should be at least 25 Hz, and the gain should be at least 60 dB. On each side, a measurement is made, and the average of both sides can be measured. Measurements were made using calipers to manually measure the distance between the two interfaces on standard B mode ultrasound. Measurement of cIMT was performed using a 3-lead ECG in the end-diastolic period.

2.5 Statistical Analysis

Version 24.0 of SPSS for Windows was used for statistical analysis. In descriptive data, patient demographics such as age, gender, IMT, and laboratory test results are presented. The data were then examined for normality by means of the Kolmogorov–Smirnov and variance homogeneity tests. This research yielded normally distributed and homogeneous data. Pearson's correlation test was used to examine the correlation between ACR and hsCRP on FMD and cIMT; $p<0.05$ is regarded as statistical significance. After conducting a bivariate test to identify the correlation between variables, a multivariate analysis with linear regression was conducted for the statistical analysis.

3. Results

3.1 Characteristics of research subjects

From all 82 research subjects, the majority were female (45 subjects (54.9%)), with an average age of 13.28±4.32 years. The mean BMI was 18.45±4.17 kg/m2. The mean systolic blood pressure was 111.67±9.73 mmHg and the average diastolic blood pressure was 73.9±4.8 mmHg. The mean ACR value was 28.94±17.64 mg/g. The mean value of hsCRP was 1.55±1.36 mmHg. From the lipid profile examination, the mean cholesterol level was 163±37.11 mg/dL, the mean HDL cholesterol level was 56.57±13.24 mg/dL, the mean LDL cholesterol level was 114.32±34.19 mg/dL, and triglyceride levels of 101.71±42.23 mg/dL.

Table 1. Baseline characteristic of research partisipants.

Variable		Total (n=82)	
		n/mean	%/SD
Demography			
	Age	13.28	4.32
	Sex		
	Male	37	45.1
	Female	45	54.9
	BMI (kg/m2)	18.45	4.17
	Sistole (mmHg)	111.67	9.782
	Diastole (mmHg)	73.9	4.806
Biomarker			
	Ureum (mg/dL)	21.7805	9.4145
	Creatinin (mg/dL)	0.5929	0.2798
	SGOT (U/L)	33.05	4.716
	SGPT (U/L)	32.89	4.751
	ACR (mg/g)	28.94	17.64
	hsCRP (mg/dL)	1.55	1.36
	HDL-C (mg/dL)	56.57	13.24
	LDL-C (mg/dL)	114.32	34.196
	TG (mg/dL)	101.71	42.23
	Kolesterol Total (mg/dL)	168.91	37.11
	Hb (g/dL)	14.11	1.22
	WBC (103/μL)	8.27	2.76
Variabilitas	Variabilitas HbA1C		
	HbA1C	43.90	34.14
	HVS	0.78	0.71
	HbA1c-SD	0.086	0.076
	HbA1c-CV	0.47	0.11
IMT (mm)		16	16
FMD (%)			

Note. All data were presented by mean SD; BMI = Body Mass Index; hsCRP = High Sensitivity CRP; HbA1C = Hemoglobin A1C; HVS = HbA1c Variability Score; ACR = Albumin to Creatinine Ratio.

HbA1c variability was calculated using 3 methods, namely the HbA1c variability score (HbA1c Variability Score/HVS), the HbA1c standard deviation (HbA1c-SD), and the HbA1c coefficient of variability (HbA1c-CV). From the examination, the mean HVS value was 43.90±34.14, the HbA1c-SD average was 0.78±0.71, and the HbA1c-CV average was 0.086±0.076). The mean thickness of the carotid artery intima-media (cIMT) for all examination subjects was 0.47 mm±0.11 mm. The mean flow-mediated Dilatation Response (FMD) of all subjects showed a mean of 16±16%.

Table 2. Data normality test with Kolmogorov-Smirnov

Variable		P
		0.134
Variable	IMT	0.075
	ACR	0.061
	hsCRP	0.826
	HDL-C	0.329
	LDL-C	0.430
	TG	0.414
	Total Kolesterol	0.177
	HVS	0.062
	HbA1c-SD	0.147
	HbA1c-CV	

Note. significant if the p-value is 0.05 or lower

3.2 Data Normality Test

Based on the Kolmogorov-Smirnov data normality test, all variables were normally distributed. BMI (p=0.134), HDL-C (p=0.826), LDL-C (p=0.329), triglycerides (p=0.430), total cholesterol (p=0.414), ACR (p=0.075), hsCRP (p=0.061), HVS (p=0.117), HbA1c-SD (p=0.062), and HbA1c-CV (p=0.147). The normality test of the data is shown in table 2.

3.3 Correlation between ACR with FMD and cIMT

Correlation test to determine the relationship between ACR and FMD. In this test, it was found that there was a statistically significant moderate correlation between ACR and FMD (r=-0.593; p=0.000). In table 5.3, based on the results of the correlation test, it was found that there was a statistically significant weak correlation between ACR and cIMT (r=0.339; p=0.002).

3.4 Correlation between hsCRP with FMD and cIMT

Meanwhile, the results of the correlation test between hsCRP and FMD showed a weak but statistically significant correlation (p=-0.375; p=0.001). There was also a statistically significant moderate correlation (r= 0.414; p=0.023) between hsCRP and and cIMT.

Table 3. Pearson's Test correlation test results.

Independent Variable	FMD		cIMT	
	r	p	r	p
Sex	0.647	0.421	0.180	0.671
Age	-0.098	0.383	0.120	0.284
ACR	-0.593	0.000	0.339	0.002
hsCRP	-0.375	0.001	0.414	0.023
Cholesterol Total	-0.167	0.134	0.240	0.030
HDL-C	0.064	0.569	0.011	0.922
LDL-C	-0.210	0.058	0.340	0.002
TG	-0.201	0.071	0.237	0.032
HVS	-0.312	0.004	0.410	0.000
HbA1c-SD	-0.339	0.002	0.279	0.011
HbA1c-CV	-0.276	0.012	0.193	0.082

Note. significant if the p-value is 0.05 or lower

From the results of the linear regression test, it was found that ACR was negatively correlated with FMD value (β=-0.360, 95%CI -0.005 - 0.000, p=0.028) and positively correlated with cIMT (β =0.442, 95%CI 0.030 - 0.623), p=0.039). Meanwhile, hsCRP was negatively correlated with FMD value (β=-0.269, 95%CI -0.036 to 0.019, p=0.043) and positively correlated with cIMT (β=0.412, 95%CI 0.021 - 0.517, p=0., 017).

Table 4. Linear regression test results

Variabel bebas	Variabel Terikat					
	FMD			cIMT		
	Beta	95% CI	p	Beta	95% CI	p
ACR	-0.360	-0.005 to 0.000	0.028	0.442	0.030 to 0.623	0.039
hsCRP	-0.269	-0.036 to .019	0.043	0.412	0.021 to 0.517	0.017

Note. significant if the p-value is 0.05 or lower; FMD = Fibromuscular dysplasia; CIMT = A carotid intima-media thickness test

4. Discussion

4.1 Correlation between ACR on FMD and cIMT

Even when ACR levels are within the normal range, microalbuminuria is the most frequently used risk marker for early renal failure in adults with T1DM. A higher ACR, even within the normal range, is associated with early atherosclerosis, according to the Adolescent Type 1 Diabetes Cardiorenal Intervention Trial (AdDIT). Adolescents enrolled in the AdDIT study with urinary albumin excretion rates in the upper tertile of the normal range showed a small but substantial increase in arterial stiffness, which predicts microalbuminuria in 85 percent of cases.[7] An early association between urinary albumin excretion and subclinical cardiovascular disease is supported. by the results of this Australian AdDIT cohort.

In this study, there was a statistically significant moderate correlation between ACR and FMD (r=-0.593; p=0.000). This finding is in line with research by El Dayem et. al. in 61 adolescents with T1DM (mean age 16.3 ± 1.5 years), showed a negative correlation between ACR and FMD (r= -0.55, p=0.0001).(8) A study on T1DM adolescents with T1DM duration of less than 5 years , T1DM had a lower mean FMD than the control group (P = 0.023), regardless of age, smoking, hypertension, or dyslipidemia. Endothelial dysfunction was found in 28 of 57 T1DM patients. FMD was lower in microalbuminuric patients (4.1%) compared to normoalbuminuric patients (10.1%, p=0.01) and controls (14.6%, p<0.001).[9] in the T1DM population, it was found that the ACR in the T1DM group with microalbuminuria was higher and statistically significant compared to the T1DM group without microalbuminuria and the control group (73.45mg/g vs 4.42mg/g vs 4.42mg/g, p<0.001). FMD in the T1DM group with and without microalbuminuria was lower and statistically significant compared to the control group (p<0.001). ACR was negatively and statistically significant correlated with FMD (r= -0.47 ± 0.22, p=0.036).[10]

Ladeia et. al. conducted a study on adolescents with T1DM (mean age 13.4 ± 3.3 years), found that microalbuminuria (ACR>30mg/g) had a negative correlation with FMD (r = -0.5, p = 0.049) even though the duration of T1DM was less than 5 years.[11] This study highlights the importance of ACR as an early marker of microalbuminuria as an early indicator of vascular disease. Consequently, the correlation between microalbuminuria and endothelial dysfunction may explain why microalbuminuria is associated with the development of extrarenal complications in T1DM patients.

This study found a statistically significant weak correlation between ACR and cIMT (r=0.339; p=0.002). In another study of 62 adolescents with T1DM with a mean age of 16.3±1.5, and a duration of 9.4±2.9 years, found that ACR, which is an early indicator of diabetic nephropathy and thus a sign of microvascular disease, is positively correlated with cIMT, which is an early sign of atherosclerosis and thus a sign of macrovascular disease.[12] The findings of this study are also consistent with the findings of Gül et. al., who found that patients with T1DM had a significantly higher cIMT than the control group, and a significant positive correlation between cIMT and microvascular problems, either nephropathy (beta=0.043, 95% CI 0.019-0.068, p=0.001) and/or retinopathy (beta=0.037, 95%CI 0.010-0.065, p=0.008).[13]

Follow-up data from the AdDIT study in adolescents with T1DM reported that higher ACR values, even within the normal range, were associated with a higher risk of developing microalbuminuria, and a poorer cardiovascular profile, as indicated by thicker cIMT during 2-4 years of follow-up in a cohort study of 546 adolescents with T1DM, assessed at ages 10-16 years.[14] Another study also found a correlation between ACR levels and increased cIMT in 60 adolescents

with T1DM with a mean age of 11.5 ± 3.5 years. (β=0.0051±0.0024, p=0.031).[15]

The presence of microalbuminuria was positively correlated with increased cIMT in adolescent patients (mean age 12±2.7 years) with T1DM. From the entire group of patients, using a univariate linear regression model, it was found that the factor that significantly correlated with cIMT was the presence of microalbuminuria (beta ± SE: 0.050 ± 0.021, p = 0.035).[16] Of the 1229 patients with T1DM who underwent ultrasound examination in the internal and common carotid arteries in 1994-1996 and again in 1998-2000 as part of the EDIC project, a long-term follow-up to the DCCT study. The group receiving intensive therapy during DCCT showed significantly less development of average cIMT than the group receiving conventional therapy. During DCCT follow-up (6.5 years), the development of cIMT thickness was associated with age, systolic blood pressure at the start of the EDIC study, smoking, LDL-C to HDL-C ratio, urinary albumin excretion rate (β=0.0912±0.0308, p= 0.003), and the mean value of glycosylated hemoglobin.[17]

Microalbuminuria is a strong and independent risk factor for cardiovascular disease in diabetics. So far, studies have contributed to establishing the association between microalbuminuria and cardiovascular disease. First, the association between urinary albumin excretion and cardiovascular disease risk did not begin at traditional microalbuminuria thresholds (i.e., ACR 2.5 mg/mmol in men and 3.5 mg/mmol in women, or equivalent urinary albumin excretion rates), but in much lower levels, ranging from ACR of 1 mg/mmol or even lower.[18] Second, the presence of microalbuminuria in diabetics has been associated with an increased risk of cardiovascular disease in a manner that is independent of urinary albumin excretion.[19]

4.2 Correlation between hsCRP on FMD and cIMT

Endothelial dysfunction, which occurs before the anatomic and clinical structural changes of atherosclerosis appear, is thought to play a role in atherogenesis. It has been demonstrated in several studies that endothelial dysfunction developed in T1DM at a young age is likely to occur before the increase in cIMT.[20-22] Gökşen et. al., studied 55 adolescents (mean age 17.7±4.0 years) with T1DM (mean duration of DM 10.4 years) found that FMD in adolescents with T1DM was lower than the healthy group of adolescents. One of the conclusions of this study is that hsCRP is an independent factor that is negatively correlated with FMD (r=-286, p=0.03).[23]

In this study, a negative correlation was found between hsCRP and FMD. hsCRP and FMD showed a weak but statistically significant correlation (p=-0.375; p=0.001). This finding is in line with the results of a study by Ohsugi et. al., who stated that in adolescents and young adults with T1DM and T2DM (age range 12 to 20 years), hsCRP was negatively correlated with FMD (β=-2.48, 95% CI (-4.6 to -0.39), p= 0.02).[24]

In this study, there was a positive correlation between hsCRP and cIMT (r= 0.414; p=0.023). Okano et. al., evaluated whether a low-grade inflammatory state contributes to the early stages of advanced carotid artery atherosclerosis in young adult T1DM patients. The results of 55 T1DM patients (22 males and 33 females, age 22.1 ± 3.6 years, duration of diabetes 14.2 ± 5.7 years) found that hsCRP levels were independently and positively correlated with mean cIMT (r = 0.429, p = 0.002).[25] This correlation finding is also in line with findings in another study where 60 T1DM patients with an average age of 14.1±2.6 years found a moderately significant positive correlation between hsCRP values and cIMT (r=0.534, p<0.001).[26]

Evidence of a correlation between hsCRP and early atherosclerotic processes in T1DM patients was also demonstrated by Atabek et al. al., where 65 children and adolescents with T1DM (33 girls and 32 boys, average age 12.7±3.8 years, with diabetes duration 6.9±3.6 years) were research subjects. From this study, it was found that hsCRP was positively and significantly correlated with CIMT (r=0.49, p=0.001).[27] In line with Atabek et. al., Research conducted by El-Asrar et. al., also found a significant correlation between hsCRP and an increase in cIMT(β= 3.020 ±0.917, p=0.001).[15]

Obesity and diabetes are associated with chronic low-grade systemic inflammatory states, which drive a vicious cycle of insulin resistance, oxidative stress, and endothelial dysfunction, which form the basis for an early and accelerated atherosclerotic process. In lean children with T1DM, low-grade systemic inflammation was also seen, which was seen in elevated levels of pro-inflammatory cytokines. Chronic systemic inflammation that begins in childhood accelerates the formation of atherosclerotic plaques and contributes to its development. Previous studies have found that children and adolescents with diabetes have higher levels of CRP, IL-6, TNF-, and leptin. In fact, levels of pro-inflammatory markers appear to be higher in lean adolescents with diabetes who have good glycemic control than in healthy adolescents.[28]

Limitation

This study has several limitations. First, the form of this research is a cross-sectional study, which cannot prove the existence of causality between the variables studied. Second, this study is a single center study, the research subjects were obtained only at the Saiful Anwar regional hospital in Malang where the subject could not represent the population of T1DM patients as a whole. Third, the number of research samples is small because the number of T1DM patients undergoing treatment both inpatient and outpatient at the Saiful Anwar Regional Hospital in Malang is still limited. Additional information can be obtained by prospective studies with a larger number of research subjects. The increase in ACR and hsCRP in T1DM patients should be investigated further in longitudinal studies with repeated samples from each patient to see if these factors are associated with the presence of endothelial dysfunction and macrovascular damage.

5. Conclusion

ACR and hsCRP correlate with the increase in cIMT and a decrease in brachial artery FMD in children and adolescent with T1DM. We conclude that initial atherosclerotic alterations may occur with altered vascular endothelial dysfunction caused by T1DM. We advise early and careful monitoring of children with T1DM to detect any changes in vascular endothelial dysfunction.

6. Declarations

6.1. Ethics Approval and Consent to participate
This study was approved by local Institutional Review Board, and all participants have provided written informed consent prior to involvement in the study.

6.2. Consent for publication
Not applicable.

6.3. Availability of data and materials
Data used in our study were presented in the main text.

6.4. Competing interests
Not applicable.

6.5. Funding source
Not applicable.

6.6. Authors contributions
Idea/concept: SW. Design: SW, NK . Control/supervision: NK, IP, CT, BS. Literature search: NK, IP, CT, BS. Data extraction: SW, NK. Statistical analysis: RP, MSR. Results interpretation: SW, NK. Critical review/discussion: NK, IP, CT, BS. Writing the article: SW, NK. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

6.7. Acknowledgements
We thank to Brawijaya Cardiovascular Research Center.

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