RESEARCH ARTICLE

# Expression of Plasma miRNA-133a is Significantly Lower in Acute Coronary Syndrome (ACS) than in Healthy/Non-ACS Subjects

Ermin Rachmawati[1,*], Djanggan Sargowo[2], Indra Wahyu Saputra[3], Riskiyah[4], Ikhwan Handirosiyanto[3], Arief Rachman Hakim[3], Mahrus Ismail[5], Tarsadi[6], Syafiq Maulana[6], Iwal Reza Ahdi[7], Alvina Puspitasari[3], Syanindita Puspa Wardhani[8]

[1]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Locari, Batu 65151, Indonesia
[2]Department of Cardiovascular, Faculty of Medicine, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia
[3]Department of Cardiovascular, Karsa Husada Hospital Batu, Jl. Ahmad Yani No.11-13, Batu 65311, Indonesia
[4]Department of Publich Health, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Locari, Batu 65151, Indonesia
[5]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Gajayana 50, Malang 65144, Indonesia
[6]Medical Bachelor Program, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Locari, Batu 65151, Indonesia
[7]Department of Internal Medicine, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Locari, Batu 65151, Indonesia
[8]Degenerative Centre, Universitas Brawijaya, Jl. Veteran, Malang 65145, Indonesia

*Corresponding author. Email: ermin.rachmawati@kedokteran.uin-malang.ac.id

Received date: Aug 4, 2024; Revised date: Oct 19, 2024; Accepted date: Oct 21, 2024

## Abstract

**BACKGROUND:** The current biomarker diagnostic modality for acute coronary syndrome (ACS), cardiac troponin, has several limitations. Emerging studies showed that micro-RNA (miR)-133a was released from infarcted heart to circulation, yet the diagnostic value of miR-133a in ACS demonstrated a conflicting result. Therefore, this study was conducted to investigate the potency of plasma miR-133a as a biomarker candidate of ACS.

**METHODS:** This was a case-controlled study, involving ACS and control subjects. The sociodemographic and clinical characteristics were assessed through medical records. A final of 39 ACS and 31 control subjects (consist of healthy and non-ACS subjects) passed the selection procedure by demonstrating a high purity of RNA. miR-133a from ACS and control subjects were detected by quantitative polymerase chain reaction (qPCR). Expression of miR-133a was evaluated for sensitivity and specificity as an ACS biomarker diagnostic using the receiver operating characteristic (ROC) curve.

**RESULTS:** Plasma miR-133a expression was stably found in ACS subjects. The plasma miR-133a level was lower in ACS than in control subjects. miR-133a effectively distinguished ACS subjects from healthy subjects (AUC=0.911) and exhibited high diagnostic performance, with a sensitivity of 87.1% and specificity of 100% at a cut-off value of 44.035. In an extended model including both control subjects (healthy and non-ACS with comorbid conditions), miR-133a maintained diagnostic significance (AUC=0.874), showing sensitivity of 76.9% and specificity of 100% at a cut-off value of 11.69.

**CONCLUSION:** Plasma miR-133a is significantly lower and effectively distinguishes ACS patients from both healthy individuals and non-ACS individuals with comorbid, with a cut-off value of 11.69. Therefore, plasma miR-133a is suggested to be a good candidate for diagnostic biomarkers of ACS.

**KEYWORDS:** circulating miRNA, miRNA-133a, acute coronary syndrome, diagnostic biomarker

***Indones Biomed J. 2024; 16(5): 464-72***

## Introduction

Acute coronary syndrome (ACS) is the leading cause of mortality worldwide, and ACS prevalence has been dramatically increased over time. The prevalence of ACS rose from 0.5% in 2013 to 1.5% in 2018 in Indonesia.(1) A large number of ACS patients continue to face delays in receiving initial therapy, which can result in life-threatening consequences such as cardiomyopathy and even heart failure. Moreover, the unit cost for currently available ACS treatment was high, thus becoming a substantial economic burden among many countries worldwide.(2,3)

Based on the American Heart Association (AHA) recommendations, the diagnosis of ACS is performed based on clinical complaints, electrocardiogram (ECG), biomarker cardiac troponin I (cTnI), and coronary angiography.(4,5) cTnI is a hallmark of laboratory diagnosis of ACS that is a sign of myocardial damage. However, most commercial cTnI immunoassays differ based on the antibody type, calibrator, and detection technique. Therefore, this may result in significant measurement variability, ambiguous medical monitoring, and risk that might affects patient treatment outcomes.(6) In addition, apparently cTnI also showed positive results in other diseases (6), making it less specific for ACS diagnosis. Atherosclerotic plaque rupture and thrombus development precede cardiac necrosis, which releases troponin.(7) However, cardiac biomarkers are not released at plaque rupture event, the time diagnostic capacity of troponin could not detect at earlier stage of ACS mechanism. Based on the background mentioned above, it is necessary to explore specific approaches before the occurrence of myocardial infarction and necrosis for the diagnosis of ACS.(8,9) One of the prime candidates in developing new diagnostic tools that may address these limitations is micro-RNA (miR) biomarkers. Recently, miRs have been proposed as potential biomarkers for use in various clinical contexts. miRs are critical effectors of gene expression suppression through post-transcriptional repression and messenger RNA (mRNA) degradation. (10,11) Interestingly, miRs are stable in circulation, has simple isolation process, and are easy to measure, making them good candidates for the diagnosis of various diseases, including ACS.(11)

Many studies have successfully found several circulating miRs that can be candidate biomarkers of ACS by their contribution to endothelial integrity, macrophage inflammatory response to atherogenic lipids, vascular smooth muscle-cell proliferation, inflammatory process that led to plaque rupture, thrombosis formation, and cardiac response because of the decrease of blood flow.(12) miR-483-5p expression increased significantly in the circulation by 0.5-1 hour after plaque rupture.(13–15) Circulation levels of miR-1 and miR-133a, were elevated and reached peak level within the first 2 h after the start of myocardial ischemia in ACS, which seem to be correlated with serum creatine kinase myocardial band (CK-MB) concentrations and cTnI levels. Increased miR-1 and miR-133a levels in the sera of patients with cardiovascular disease indicate the existence of myocardial damage, loss of myocardial contractility and activated platelets by ischemia.(16) Even with the suggested potential as biomarker for ACS, unfortunately, there is lack of studies investigating these miR profiles in ACS patients carried out in Indonesia. Therefore, this study was conducted to investigate the profile of miR-483-5p, miR-133a, and miR-1 as biomarker candidates of ACS in Indonesia.

## Methods

### Study Design and Participant Selection

This research was a case-controlled analytical observational study which was conducted in January-July 2024. The research participants were recruited from the emergency room of Karsa Husada Hospital Batu, Indonesia. Epi InfoTM version 7.2 b (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used for sample size calculation public domain software, using unmatched case-control study considering a ratio of controls to cases of 1:1, 40% per cent of control exposed, and odd ratio of 4.18 according to previous study.(17) The minimum sample size calculated for this study was 33 subjects for each ACS and control group.

The subject's inclusion criteria were determined based on the European Society of Cardiology (ESC) and American College of Cardiology (ACC) guideline, including: 1) experienced chest pain episode of less than 24 hours; 2) first-time episodes; and 3) complete clinical data. The subjects were excluded from the selection process if met several criteria including: 1) taken immunosuppressants or immune enhancers; 2) diagnosed with other cardiac insufficiency, hematologic disease, renal failure disease, malignancy, or autoimmune disease. Subjects in the control group were selected based on the absence of clinical ACS, and then further classified into healthy control subjects (without any comorbid) and non-ACS subjects (subjects without ACS but suffered from one or more comorbidities such as ACS

history, hypertension, type 2 diabetes mellitus (T2DM), or dyslipidaemia). This protocol of this study was approved by Medical Ethics Committee from Karsa Husada Hospital (Registration No. 020/2207/102 13/2023).

**Baseline Characteristic Assessment of Study Subjects**

Sociodemographic profiles including age, sex, educational background, occupation and baseline clinical characteristics such as blood lipid, blood sugar, blood pressure, heart rate, body mass index (BMI), past medical history, and type of ACS were taken from subjects' medical records.

**Blood Sample and Plasma Collection**

Five mL of blood was drawn aseptically from a well-identified antecubital vein in the forearm immediately after the patient's admission to the emergency room with suspected ACS from a typical chest pain complaint. Blood was stored in EDTA tubes, then kept in an open chamber until plasma and cells were separated using centrifugation for 30 minutes at 5000 rpm. The supernatant was taken and stored at -70°C.

**miR Isolation**

The miR was extracted using Maxwell RSC miRNA Plasma and Serum Kit Promega (Cat. No. AS1460; Promega, Madison, WI, USA). Briefly, after mixed 100 µL of Nuclease-Free Water, 230 µL of Lysis Buffer C and 80 µL of Proteinase K to the CW Spin Basket, the samples were incubated at 37°C for 15 minutes and centrifuged at maximum speed for 5 minutes to collect lysate at the bottom of the tube. Subsequently, the full lysate was transferred to well which was followed by addition of 50 µL of Nuclease-Free Water, a plunger, 10 µL of blue DNase I Solution.(18)

**Addition of Poly-A Tail and cDNA Synthesis**

The poly-A tail addition was carried out prior to the cDNA synthesis using Escherichia coli Poly(A) Polymerase (Cat. No. M0276; New England Biolab, Ipswich, MA, USA). The synthesis of cDNA followed the manual instruction of the kit from Go-Script Reverse Transcription System (Cat. No. A1560; Promega). Briefly, The RNA was mixed and centrifuged with primer oligo-DT and Nuclease-Free Water, then stored at 70°C heat block for 5 minutes. Immediately, the RNA was chilled in ice water for 5 minutes. The reverse transcription reaction mix consisted of GoScript 5x Reaction Buffer, MgCl2, Polymerase Chain Reaction (PCR) Nucleotide mix, and GoScript Reverse Transcriptase. The annealing process was performed in a heat block at 25°C for 5 minutes, followed by extension which was run in a heat block at 42°C for up to one hour. Before proceeding with quantitative PCR (qPCR), the reverse transcriptase was inactivated in a heat block at 70°C for 15 minutes. For long-term storage, the cDNA stored at -20°C.(18)

**miR Quantification using Real Time PCR (RT-PCR)**

U6 was used as an internal control. The detail sequences of each primer gene were as follow: miR-483-5p forward, 5'-GCCGAGAAGACGGGAGGAAA-3'; miR-483-5p reverse, 5'-CTCAACTGGTGTCGTGGA-3'; miR-133 forward, ACA CTC CAG CTG GGT TTG GTC CCC TTC AAC; miR-133 reverse, CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG CAG CTG GT; miR-1 forward, ACA CTC CAG CTG GGT GGA ATG TAA AGA AGT; miR-1 reverse, TCA ACT GGT GTC GTG GAG TCG GCA ATT CTT GAG CAG CTG GT; U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; and U6 reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

GoTaq qPCR master mix (Catalogue No. A6001; Promega) was added with forward, reverse primer, and Nuclease-Free Water to a final volume of 20 µL. Next, the cDNA was added to this reaction mix. The PCR was conducted in 40 cycles including: GoTag DNA polymerase activation cycle 1 at 95°C for 2 minutes; and denaturation for 15 seconds at 95°C, annealing at 60°C for 1 minutes, extension at 60°C for 1 minutes for 40 cycles. The relative expression of each miR was calculated based on the threshold cycle (CT), and the values were normalized to U6 and expressed as $2^{-(Ct[miR-133a]-Ct[U6])}$.(16)

Three miRs were selected from literature in the preprocessing steps. The identification of these miR in 8 ACS subjects was carried out using RT-PCR technique after primer optimization and normalization. Subsequently, the miRs which showed a number of counts above zero point in at least 80% samples (n=6) were used for the biomarker analysis. Only miR-133a exhibited stable expression in 8 ACS subjects, thus used for the further analysis (Figure 1). Additionally, miR-1 and miR-483-5p were used as positive control in 5 healthy control and 5 non-ACS control subjects.

**Data Analysis**

Data for miR expression, sociodemographic and clinical characteristic variables were presented using descriptive statistics, where the numerical data presented as mean and standard deviation (SD) while the categorical data presented as frequency and percentage. The association between clinical characteristic parameters with type of ACS were analysed using Chi-Square test. Statistical tests to provide evidence about sensitivity, specificity, area under the curve

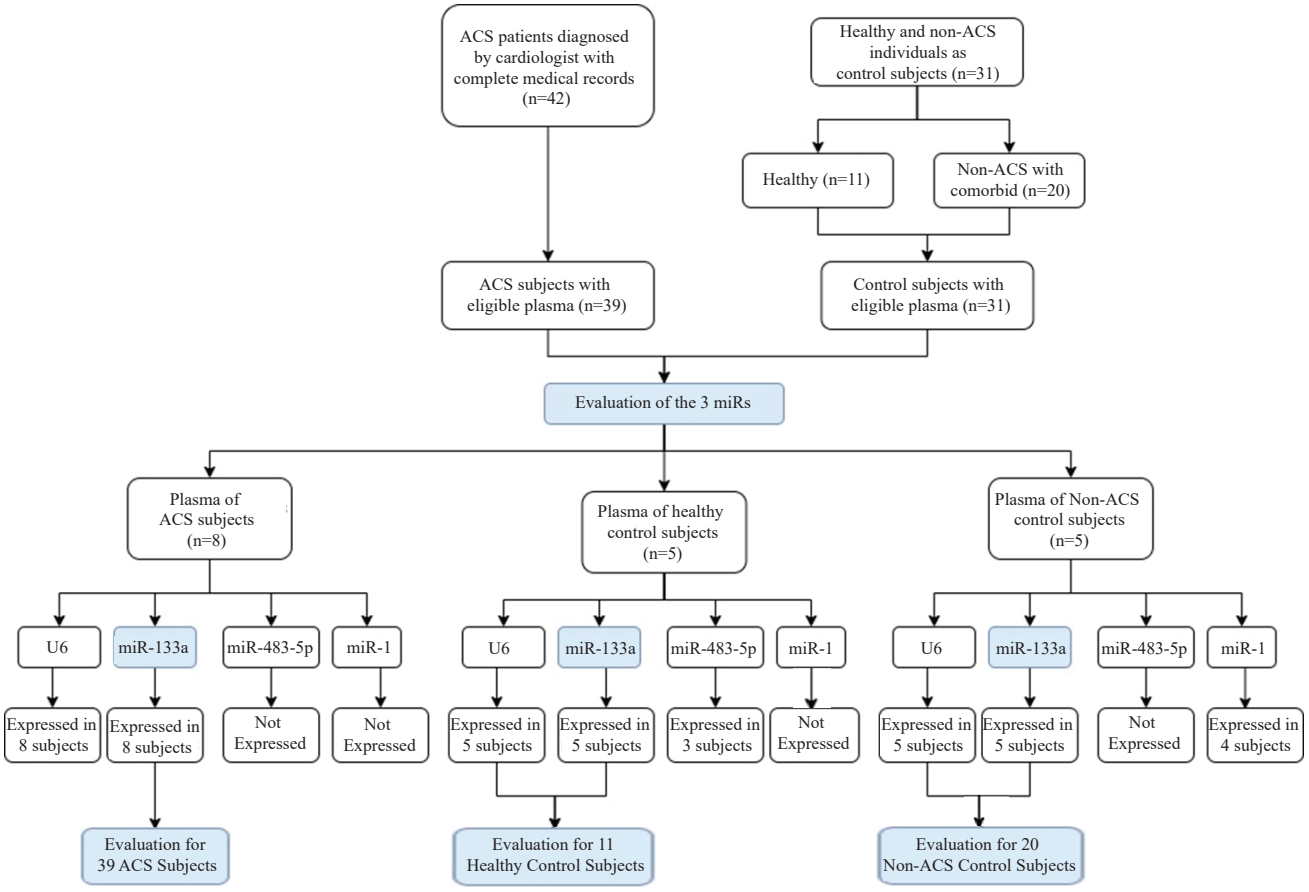


**Figure 1. Flowchart of subject selections and miR analysis procedures.** Forty-two ACS patients had complete data in their medical records. Three plasma patients were not used as samples due to the blood lysis and the storage of>24 hours. Therefore, only 39 ACS subjects and 31 control subjects signed informed consent and enrolled in this study. Housekeeping gene U6 was consistently expressed in 8 ACS subjects' plasma. Only miR-133 was stably expressed in 8 subjects. Meanwhile, miR-483-5p was expressed in healthy control subjects, and miR-1 was detected in the circulation of non-ACS control subjects with comorbidity.

(AUC), the receiver operating characteristic (ROC) curve of miR-133a as a diagnostic candidate for ACS were analysed with SPSS software v 26.0 (IBM Corporation, Armonk, NY, USA).

## Results

### The Subjects Selections

Patients diagnosed by cardiologists were included as ACS subject candidates. Initially, there were 42 ACS patients with complete medical records data required in this study, however only 39 final subjects had plasma that met the quality for miRNA examination. Therefore 39 ACS subjects and 31 control subjects were enrolled. The control subjects further classified as healthy control subjects (n=11) as well as non-ACS subjects with one or more comorbidities (n=20) (Figure 1).

### Study Subjects' Demographic and Baseline Clinical Characteristics

Most of the ACS subjects were male (66.7%). The predominant age who experienced ACS were 45-65 years old (71.7%). Lower education tended to increase the risk of ACS (Table 1). In ACS subjects, the highest frequency of comorbid found in subjects was hypertension (39%) followed by dyslipidaemia (24.4%). Meanwhile, in control subjects the most found comorbid was also hypertension (40.3%) but followed by T2DM (25%).

Most of the participants involved in this study had at least 1 comorbid (46.2%). At the admission room in the emergency department, the subjects had a variety of systolic pressure. Most subjects had a normal heart rate (Table 2). The lipid profile of subjects assessed at the Emergency Department characterized by high low-density lipoprotein (LDL), triglyceride (TG), low high-density lipoprotein (HDL), and normal cholesterol level. Moreover, there was

**Table 1. Sociodemographic characteristic of the ACS and the control subjects.**

| Category | Frequency [n (%)] | |
|---|---|---|
| | ACS Subjects (n=39) | Control Subjects (n=31) |
| Sex | | |
| Male | 26 (66.7) | 11 (35.5) |
| Female | 13 (33.3) | 20 (64.5) |
| Age (years old) | | |
| 36-45 | 3 (7.7) | 10 (32.3) |
| 45-55 | 12 (30.7) | - |
| 56-65 | 16 (41.0) | 11 (35.5) |
| >65 | 8 (20.5) | 10 (32.3) |
| Educational Background | | |
| Elementary | 8 (20.5) | 1 (3.2) |
| Junior High School | 11 (28.2) | 3 (9.7) |
| Senior High School | 16 (41.0) | 9 (29.0) |
| Diploma | - | - |
| Bachelor | 4 (8.5) | 18 (58.0) |
| Magister/Doctoral | - | |
| Occupation | | |
| Self-employed | 18 (46.1) | 12 (41.9) |
| Driver | 1 (2.56) | - |
| Farmer | 9 (23.1) | 1 (3.2) |
| Teacher | 1 (2.5) | 1 (3.2) |
| Civil attendance | 1 (2.5) | - |
| Labour | 2 (5.1) | 10 (32.3) |
| House wife | 7 (17.9) | 7 (25.8) |
| Sum of Past History | | |
| No Comorbid | 10 (25.6) | 10 (32.3) |
| 1 Comorbid | 18 (46.2) | 2 (6.5) |
| 2 Comorbid | 9 (23.1) | 7 (22.6) |
| 3 Comorbid | 2 (5.1) | 12 (38.7) |
| Type of Comorbid | | |
| Hypertension | 16 (39.0) | 21 (40.3) |
| T2DM | 9 (21.9) | 13 (25.0) |
| Coronary Artery Disease | 7 (17.0) | 10 (19.2) |
| Dyslipidaemia | 10 (24.4) | 8 (15.4) |
| Heart Failure | 8 (19.5) | - |

Sum of past history includes: ACS history, hypertension, T2DM, dyslipidaemia.

association between TC with the type of ACS ($p$=0.042). Meanwhile, HDL ($p$=0.078), HR ($p$=0.129), LDL ($p$=0.197), and TG ($p$=0.670) were not significantly correlated with ACS.

**miR-133a was Found in the Plasma of ACS Subjects**

It was found that miR-133a was expressed in the plasma of ACS and control subjects. The data showed that plasma miR-133a was down-regulated in ACS subjects. miR-483-5p and miR-1 were not expressed in ACS subjects. However, the miR-483-5p was expressed in 3 healthy control subjects with the CT value of 34.14, 34.64, and 37.53, respectively. While, the CT value of miR-1 expression which was expressed in 4 non-ACS control subjects were 35.25, 35.95, 37.1, and 37.82, respectively.

**Plasma miR-133a As a Sensitive Predictor for ACS**

Two model of ROC was carried out to determine the sensitivity and specificity of miR-133a as biomarker diagnostic of ACS. In the first model, 11 healthy control and 39 ACS subjects were involved in the analysis. The results of the independent sample t-test showed a homogeneity value of 0.131 with a significance of 0.000, so it was concluded that there was a difference in the value of miR-133a between groups of healthy control and ACS subjects. The ROC curves of miR-133a with this model revealed the probability of this miR as a valuable marker for ACS. The ROC analysis revealed that miR-133a had a sensitivity of 87.1% and a specificity of 100% for diagnosing ACS, using a cut-off value of 44.035. The diagnostic value of miR-133a was high, with an area under the ROC curve (AUC) of 91.1% (95% CI: 0.829–0.994, $p$=0.000) (Figure 2).

In the second model, all control subjects consist of the healthy control and the non-ACS control were included. Therefore, total 31 control (11 healthy control + 20 non-ACS control with comorbid) and 39 ACS subjects were involved in the analysis. The results of the independent sample t-test showed that there was a significant difference in miR-133a concentration ($p$=0.024) between ACS and both control subjects in this model. The results of the ROC analysis from model 2 showed that the sensitivity value of miR-133a in the diagnosis of ACS was 76.9% and the specificity was 100%, based on a cut-off value 11.69. The miR-133a diagnostic value was high (AUC=87.4% 95% CI=0.784–0.963), $p$=0.000) (Figure 3).

## Discussion

The results of this current study support previous studies that men were four times more likely to experience ACS than women.(19–21) The age distribution of ACS patients tends to shift to younger ages due to sedentary lifestyles.(22–24) The educational background of most patients was in elementary, junior and high school, which suggested low awareness and knowledge regarding ACS.(25,26) The current findings confirmed that dyslipidaemia, especially total cholesterol, hypertension, T2DM, heart failure are the risk factors for ACS.(27)

**Table 2. Baseline characteristic of the ACS subjects (n=39).**

| Parameters | Frequency [n (%)] | Mean±SD |
|---|---|---|
| Body Mass Index (kg/m²) | | 23.46±2.35 |
| Normal (18.5-24.9) | 28 (71.8) | |
| Overweight (25-29.9) | 11 (28.2) | |
| Obesity (>30) | - | |
| Systolic Blood Pressure (mmHg) | | 135.67±28.89 |
| Normal (<120) | 12 (30.8) | |
| Pre-Hypertension (120-139) | 12 (30.8) | |
| Hypertension stage 1 (140-159) | 8 (20.5) | |
| Hypertension stage 2 (≥160) | 7 (17.9) | |
| Diastolic Blood Pressure (mmHg) | | 81.87±12.54 |
| Normal (<120) | 19 (48.7) | |
| Pre-Hypertension (120-139) | 11 (28.2) | |
| Hypertension stage 1 (140-159) | 5 (12.8) | |
| Hypertension stage 2 (≥160) | 4 (10.3) | |
| Heart Rate (bpm) | | 79.54±15.47 |
| Normal | 34 (87.2) | |
| Tachycardia | 5 (12.8) | |
| Cholesterol (mg/dL) | | 167.13±46.64 |
| Normal (<200) | 26 (66.7) | |
| Middle (200-239) | 12 (30.8) | |
| High (>239) | 1 (2.6) | |
| Triglyceride (mg/dL) | | 120.59±49.45 |
| Normal (<150) | 31 (79.5) | |
| Middle (150-199) | 4 (10.3) | |
| High (>199) | 4 (10.3) | |
| LDL-C (mg/dL) | | 108.±75.63 |
| Normal (<130) | 26 (66.7) | |
| Middle (130-159) | 12 (30.8) | |
| High (>159) | 1 (2.6) | |
| HDL-C (mg/dL) | | 46.98±38.93 |
| High (>60) | 4 (10.3) | |
| Normal (40-60) | 17 (43.5) | |
| Low (<40) | 18 (46.2) | |
| Blood Sugar Levels (mg/dL) | | 113.53±43.95 |
| Normal (≤100) | 18 (46.2) | |
| Pre-Diabetes (101-125) | 13 (33.3) | |
| Diabetes (≥126) | 8 (20.5) | |
| cTnI (mmol/L) | | 6.28±22.92 |
| CK-MB (mmol/L) | | 17.89±38.99 |
| Diagnosis | | |
| STEMI | 13 (33.3) | |
| NSTEMI | 23 (58.9) | |
| UAP | 3 (7.7) | |

The diagnosis of ACS in patients with typical or atypical chest pain by cTnI as the gold standard for diagnosis of ACS with 99$^{th}$ percentile values is not without its drawbacks. Early diagnosis may be hampered by the fact that cardiac troponins may not always be increased at the beginning of myocardial infarction. Furthermore, diseases other than ACS can also cause alterations in troponin levels, such as acute inflammatory myocarditis, skeletal myopathies, acute pulmonary embolism, hypertensive crisis, and cerebrovascular accidents.(28,29) Thus, misinterpretation of detectable troponin levels might result in incorrect therapy selection and diagnosis confusion. In addition, it is difficult to define a standard cut-off, especially for cTnI, due to the large number of commercially available tests with different characteristics and 99$^{th}$ percentiles. Furthermore, 99$^{th}$ percentile concentrations are strongly influenced by population demographics such as age and gender; which currently, these factors have not been standardized.(6)

Due to the ease of detection in circulation, stable nature, and production triggered by myocardial stress or thrombosis formation, three miRs, including miR-1, miR-483-5p and miR-133a, are proposed as novel biomarkers for the diagnosis of ACS. Interestingly, in this study, expressions of miR-483-5p and miR-1 were not detected in ACS patients. The fact that miR-1 is not only secreted by injured cardiomyocytes (6,30), but also by the activated platelets (31), as well as the enrolled patients received antiplatelet drug was one of the possible contributing reasons that miR-1 expression was not detected.

Only miR-133a was expressed in ACS patient's circulation. This study's findings contradicted other research that reported an increase in circulating miR-133a (32) following myocardial infarction as this study demonstrated a reduction in plasma miR-133a in ACS patients. Furthermore, myocardial necrosis caused by hypoxia in ACS condition led to miR-133a dysfunction by alteration in expression or post transcriptional level which might trigger arrhythmia. miR-133a was also down-regulated in hypoxic cardiomyocyte due to coronary obstruction.(33) Other evidence showed that ischemic reperfusion injury increased apoptosis which was caused by lower expression of miR-133a, the apoptosis suppressor.(34) Moreover, lower cardiac miR-133a in ACS patients was correlated with the presence of ventricular fibrillation.(35) Consequently, the decrease of cardiac miR-133a in ACS patients is suggested to relate with the reduced level of circulating miR-133a. This present study showed that most ACS patients had hypertension and few of them suffer from heart failure. *In vivo* and human studies have reported lower circulating miR-133a levels in animals or patients with left ventricular hypertrophy (LVH), a condition associated with hypertension and heart failure. (36,37) Therefore, it can be proposed that the patients in this study might experience the LVH which was not observed.

Two model of ROC curve for miR-133a demonstrated a high diagnostic value of miR-133a.(38–40) Thus, while

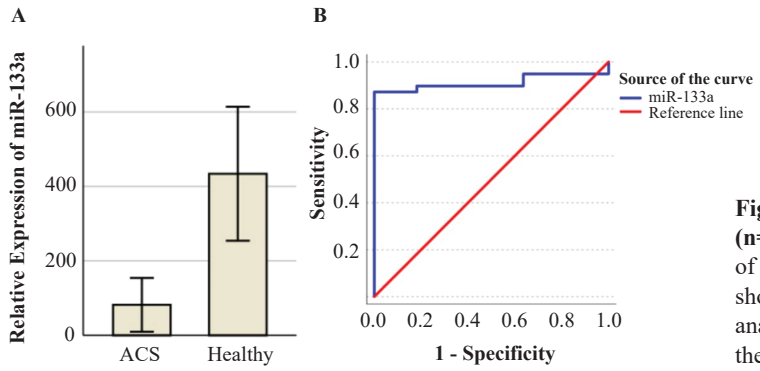


**Figure 2. The plasma miR-133a profile between ACS (n=39) and healthy control (n=11) subjects.** A: Histogram of miR-133 profile in ACS and healthy control subjects, showed significant difference ($p$<0.05). B: ROC curve analysis of miR-133a. The blue line indicated the miR, and the red line indicated the reference.

plasma miR-133a shows potential as a biomarker for ACS, its diagnostic accuracy may be influenced by the presence of comorbid conditions. The results of this study showed that the level of miR-133a in non-ACS individuals with comorbid is lower than the healthy individuals. Compared to troponin which has low specificity (29), miR-133a was suggested to have better specificity. miR-133a can be used accurately to determine non-ACS patients by confirming negative test results in healthy individuals or those with diseases other than ACS.

Since miRNA levels can fluctuate, serial measurements of miRNA-133a over time should be conducted. Unfortunately, in this study, miRs were only measured once. The timing of blood collection relative to the onset of chest pain was also not precisely documented. Although this study is the first to demonstrate the downregulation of plasma miR-133a in ACS patients, the specificity of these miRNAs was compromised, suggesting they may not provide additional information beyond other sensitive ACS markers. Therefore, future recommendations include validating these findings with animal experiments to explore the differences in miR-133a profiles in cardiomyocytes versus circulation. As this is the first study of its kind conducted in Indonesia, expanding the sample size, carry out miR sequencing with the bioinformatic analysis and considering a multicentre approach would strengthen the results. Additionally, developing applications to precisely detect the onset of chest pain could serve as a critical benchmark for clinical research. While the results presented in this manuscript offer potential for alternative biomarkers, the findings are still preliminary and require further validation.

## Conclusion

In conclusion, miR-133a was expressed in ACS patients' circulation and was down-regulated compared to both healthy individuals and non-ACS individuals with comorbid, with the cut-off value of 11.69. Therefore, plasma miR-133a is suggested to be a good candidate for diagnostic biomarkers of ACS.

## Acknowledgments

We extend our sincere gratitude to our field crew at the emergency room and Cardiovascular Care Unit (CVCU) Karsa Husada Hospital, particularly to Alvina Puspitasari for her tireless efforts in gathering data. The assistance and participation of the study participants, without whom this

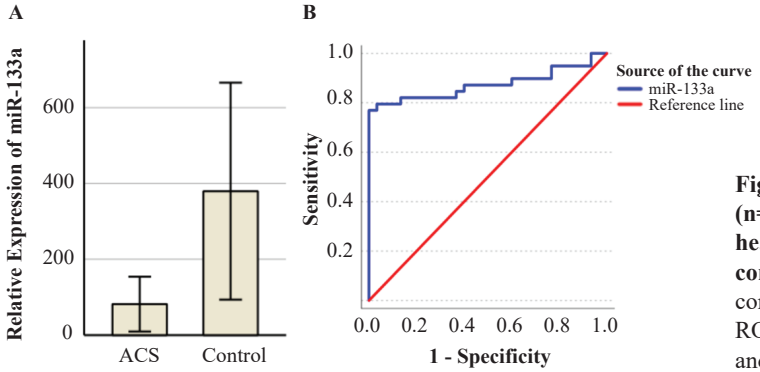


**Figure 3. The plasma miR-133a profile between ACS (n=39) and control (n=31) subjects (including both healthy control subjects and non-ACS subjects with comorbid).** A: Histogram of miR-133 profile in ACS and control subjects, showed significant difference ($p$<0.05). B: ROC curve of miR-133a. The blue line indicated the miR, and the red line indicated the reference.

research would not have been feasible, is also something we would like to acknowledge.

We are also deeply thankful to Prodia Research Centre Grant 2023 and DIPA LP2M Universitas Islam Negeri Maulana Malik (Registration No. 025.04.2.423812/2024; November 24, 2023) for the financial support.

## Authors Contribution

ER and DS were involved in concepting and planning the research, IWS, ARH, HR and AP performed the data acquisition/collection, SM, TT, MI and SPW calculated the experimental data and performed the analysis, ER, SM, TT, RR, and IRA drafted the manuscript and designed the figures, aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

## References

1. Juzar DA, Muzakkir AF, Ilhami YR, Taufiq N, Astiawati T, Junior IM, *et al.* Management of acute coronary syndrome in Indonesia: Insight from one ACS multicenter registry. Indones J Cardiol. 2022; 43(2): 45–55.
2. Salari N, Morddarvanjoghi F, Abdolmaleki A, Rasoulpoor S, Khaleghi AA, Hezarkhani LA, *et al.* The global prevalence of myocardial infarction: a systematic review and meta-analysis. BMC Cardiovasc Disord. 2023; 23(1): 206. doi: 10.1186/s12872-023-03231-w.
3. Uli RE, Satyana RPU, Zomer E, Magliano D, Liew D, Ademi Z. Health and productivity burden of coronary heart disease in the working Indonesian population using life-table modelling. BMJ Open. 2020; 10(9): e039221. doi: 10.1136/bmjopen-2020-039221.
4. Faramand Z, Helman S, Ahmad A, Martin-Gill C, Callaway C, Saba S, *et al.* Performance and limitations of automated ECG interpretation statements in patients with suspected acute coronary syndrome. J Electrocardiol. 2021; 69S: 45–50.
5. Suzuki K, Komukai K, Nakata K, Kang R, Oi Y, Muto E, *et al.* The usefulness and limitations of point-of-care cardiac troponin measurement in the emergency department. Intern Medi. 2018; 57(12): 1673–80.
6. Park KC, Gaze DC, Collinson PO, Marber MS. Cardiac troponins: From myocardial infarction to chronic disease. Cardiovasc Res. 2017; 113(14): 1708–18.
7. Meiliana A, Wijaya A. The Changing face of atherosclerosis. Indones Biomed J. 2023; 15(3): 194–221.
8. Tanase DM, Gosav EM, Costea CF, Ciocoiu M, Lacatusu CM, Maranduca MA, *et al.* The intricate relationship between type 2 diabetes mellitus (T2DM), insulin resistance (IR), and nonalcoholic fatty liver disease (NAFLD). J Diabetes Res. 2020; 2020: 3920196. doi: 10.1155/2020/3920196.
9. Califf RM. Biomarker definitions and their applications. Exp Biol Med. 2018; 243(3): 213–21.
10. Liebetrau C, Möllmann H, Dörr O, Szardien S, Troidl C, Willmer M, *et al.* Release kinetics of circulating muscle-enriched microRNAs in patients undergoing transcoronary ablation of septal hypertrophy. J Am Coll Cardiol. 2013; 62(11): 992–8.
11. Li S, Lee C, Song J, Lu C, Liu J, Cui Y, *et al.* Circulating microRNAs as potential biomarkers for coronary plaque rupture. Oncotarget. 2017; 8(29): 48145–56.
12. Meiliana A, Wijaya A. MicroRNAs in lipid metabolism and atherosclerosis. Indones Biomed J. 2014; 6(1): 3–16.
13. Zhao Y, Song X, Ma Y, Liu X, Peng Y. Circulating mir-483-5p as a novel diagnostic biomarker for acute coronary syndrome and its predictive value for the clinical outcome after PCI. BMC Cardiovasc Disord. 2023; 23(1): 360. doi: 10.1186/s12872-023-03387-5.
14. Zhu H, Liang H, Gao Z, Zhang X, He Q, He C, *et al.* MiR-483-5p downregulation alleviates ox-LDL induced endothelial cell injury in atherosclerosis. BMC Cardiovasc Disord. 2023; 23(1): 521. doi: 10.1186/s12872-023-03496-1.
15. Zhang H, Zhang Z, Liu Z, Mu G, Xie Q, Zhou S, *et al.* Circulating miR-320a-3p and miR-483-5p level associated with pharmacokinetic–pharmacodynamic profiles of rivaroxaban. Hum Genomics. 2022; 16(1): 72. doi: 10.1186/s40246-022-00445-5.
16. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, *et al.* Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circ Cardiovasc Genet. 2011; 4(4): 446–54.
17. Dan-Nwafor CC, Ogbonna U, Onyiah P, Gidado S, Adebobola B, Nguku P, *et al.* A cholera outbreak in a rural north central Nigerian community: An unmatched case-control study. BMC Public Health. 2019; 19(1): 112. doi: 10.1186/s12889-018-6299-3.
18. Le MN, Nguyen TA. Innovative microRNA quantification by qPCR. Mol Ther Nucleic Acids. 2023; 31: 628–30.
19. Altaf A, Shah H, Salahuddin M. Gender based differences in clinical and angiographic characteristics and outcomes of acute coronary syndrome (ACS) in Asian population. Pak J Med Sci. 2019; 35(5): 1349–54.
20. Pagidipati NJ, Peterson ED. Acute coronary syndromes in women and men. Nat Rev Cardiol. 2016; 13(8): 471–80.
21. Winzer EB, Woitek F, Linke A. Physical activity in the prevention and treatment of coronary artery disease. J Am Heart Assoc. 2018; 7(4): e007725. doi: 10.1161/JAHA.117.007725.
22. Duran AT, Ewing Garber C, Cornelius T, Schwartz JE, Diaz KM. Patterns of sedentary behavior in the first month after acute coronary syndrome. J Am Heart Assoc. 2019; 8(15): e011585. doi: 10.1161/JAHA.118.011585.
23. Anthony N, Hassan A, Ghani U, Rahim O, Ghulam M, James N, *et al.* Age-related patterns of symptoms and risk factors in acute coronary syndrome (ACS): A study based on cardiology patients' records at Rehman Medical Institute, Peshawar. Cureus. 2024; 16(4): e58426. doi: 10.7759/cureus.58426.
24. Indah AA, Ramadhan P V, Pratama V. Acute coronary syndrome in well trained young adult: A rare cases. Cardiovasc Cardiometabol J. 2020; 1(2): 69–76.
25. Lakew BA, Bekele DM, Alemu Kassa W, Dugassa Tolera B. Suboptimal knowledge and care of patients with acute coronary syndrome among nurses working in selected hospitals in Addis Ababa, Ethiopia 2021. Vasc Health Risk Manag. 2023; 19: 637–49.
26. Al-Zakwani I, M Mabry R, Zubaid M, Alsheikh-Ali AA, Almahmeed W, Shehab A, *et al.* Association between education and major adverse cardiac events among patients with acute coronary syndrome in the Arabian Gulf. BMJ Glob Health. 2019; 4(1): e001278. doi: 10.1136/bmjgh-2018-001278.
27. Lauder L, Mahfoud F, Azizi M, Bhatt DL, Ewen S, Kario K, *et al.* Hypertension management in patients with cardiovascular comorbidities. 2023; 44(23): 2066–77.

28. Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, *et al.* Sensitive troponin I assay in early diagnosis of acute myocardial infarction. N Engl J Med. 2009; 361(9): 868–77.
29. Yang G, Yao Y, Du Y, Huang J. Cardiac troponin had limited diagnostic value for acute myocardial infarction in renal insufficiency: A meta-Analysis. Biomark Med. 2020; 14(6): 481–93.
30. Pikir BS, Andrianto, Suryawan IGR, Hermawan HO, Kartikasari DP, Harsoyo PM. microRNA-1 induces transdifferentiation of peripheral blood CD34+ cells into cardiomyocytes-like cells. Indones Biomed J. 2022; 14(3): 269–75.
31. Gidï O, Van Der Brug M, Jenny¨ohman JJ, Gilje P, Bj¨ B, Olde B, *et al.* Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. Blood. 2013; 121(19): 3908–17, S1–26.
32. Xiao Y, Zhao J, Tuazon JP, Borlongan C V, Yu G. MicroRNA-133a and myocardial infarction. Cell Transplant. 2019; 28(7): 831–8.
33. Li A ying, Yang Q, Yang K. miR-133a mediates the hypoxia-induced apoptosis by inhibiting TAGLN2 expression in cardiac myocytes. Mol Cell Biochem. 2015; 400(1–2): 173–81.
34. He B, Xiao J, Ren AJ, Zhang YF, Zhang H, Chen M, *et al.* Role of miR-1 and miR-133a in myocardial ischemic postconditioning. J Biomed Sci. 2011; 18(1): 22. doi: 10.1186/1423-0127-18-22.
35. Boštjančič E, Brandner T, Zidar N, Glavač D, Štajer D. Down-regulation of miR-133a/b in patients with myocardial infarction correlates with the presence of ventricular fibrillation. Biomed Pharmacother. 2018: 99: 65–71.
36. Wen P, Song D, Ye H, Wu X, Jiang L, Tang B, *et al.* Circulating MiR-133a as a biomarker predicts cardiac hypertrophy in chronic hemodialysis patients. PLoS One. 2014; 9(10): e103079. doi: 10.1371/journal.pone.0103079.
37. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, Van Der Made I, *et al.* MiR-133 and miR-30 regulate connective tissue growth factor: Implications for a role of micrornas in myocardial matrix remodeling. Circ Res. 2009; 104(2): 170–8.
38. Wexler Y, Nussinovitch U. The diagnostic value of Mir-133a in ST elevation and non-ST elevation myocardial infarction: A meta-analysis. Cells. 2020; 9(4): 793. doi: 10.3390/cells9040793.
39. Liu G, Niu X, Meng X, Zhang Z. Sensitive miRNA markers for the detection and management of NSTEMI acute myocardial infarction patients. J Thorac Dis. 2018; 10(6): 3206–15.
40. Peng L, Chun-guang Q, Bei-fang L, Xue-zhi D, Zi-hao W, Yun-fu L, *et al.* Clinical impact of circulating miR-133, miR-1291 and miR-663b in plasma of patients with acute myocardial infarction. Diagn Pathol. 2014; 9: 89. doi: 10.1186/1746-1596-9-89.