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ASSOCIATION OF CIRCULATING MIRNAS-183-5P AND 208B WITH ACUTE CORONARY SYNDROME

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Abstract

Background: Acute coronary syndromes (ACS) is the highest risk of fatality worldwide, cardiac troponins and creatine kinase are widely used clinically in diagnosis of ACS, but they have some limitations .Thus, there is a need to find a novel biomarker with higher accuracy. This work aimed to assess the predictive potential of serum miR-183-5p and miR-208b expression in acute coronary syndrome. **Method:** The expression levels of serum miRNA-183-5p and miRNA-208b were evaluated by quantitative real-time polymerase chain reaction in 52 patients with acute myocardial infarction (AMI), 23 unstable angina (UA) patients, and 25 healthy volunteers. **Results:** Mir-183-5p and miR -208b expression levels were significantly upregulated in serum of unstable angina and AMI patients compared to normal controls ($p<0.001$). In addition, only miR-208b expression levels was over-expressed in unstable angina patients compared to the control group ($p=0.044$). Mir-183-5p had diagnostic value for AMI at cut off point of ≥8.03 with a sensitivity and specificity of 92% and 73%, respectively. While, miR- 208b had sensitivity of 96% and specificity of 60% at a cut-off point of ≥2.09. **Conclusion:** Mir-183-5p could be used as a non-invasive biomarker to that could detect ACS early and discriminate AMI patients and this could improve health outcome.

Keywords: acute coronary syndrome; AMI; miR-208b; miR-183-5p

INTRODUCTION

Worldwide, acute coronary syndromes (ACS) are estimated to be the leading cause of death and loss of disability-adjusted life years in both developing and developed countries

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[1, 2]. ACS occurs because of myocardial ischemia after unstable coronary atherosclerotic plaque formation, and its clinical presentations include acute myocardial infarction (AMI) and unstable angina (UA) [3]. AMI might lead to defects in functioning myocytes resulting in myocardial fibrosis and left ventricle dilatation [4].

Commonly, cardiac troponins, and creatine kinase brain/muscle subtype (CK-MB) have been used as biomarkers to diagnose and assess the prognosis of AMI but have certain limitations. Cardiac muscle isoform of troponin T (cTnT). Is an ~36-kDa protein consisting of 297 amino acids including the first methionine with an isoelectric point (pI) of 4.88. It is the tropomyosin- binding and thin filament anchoring subunit of the troponin complex in cardiac muscle cells. TNNT2 gene is expressed in vertebrate cardiac muscles and embryonic skeletal muscles.[8][9][10] It begins to rise within three to four hours, and CK-MB first appears four to six hours after the onset of myocardial injury. These biomarkers are well known to be increased in patients with chronic kidney disease (CKD) and can be misleading even in the absence of clinically suspected myocardial ischemia [5]. **MicroRNAs (miRNAs) are endogenous, small (22–24- nucleotide) noncoding RNA molecules that regulate the expression of mRNA by combining with the 3′-untranslated region (3′-UTR), subsequently triggering the degradation of mRNA or having negative effects on transcription . miRNAs can regulate nearly 60% of coding genes to exert their biological functions [6] . Recently, many studies have shown that miRNAs can regulate endothelial dysfunction, inflammation, cell autophagy, platelet activation, and aggregation [7–9]. Moreover, miRNA expression can affect the stability of atherosclerotic plaques. miRNAs possess tissue-specific expression and can be secreted into blood or urine. MiRNAs of circulatory system can be used as biomarkers of diagnosis, treatment, and prevention of diseases, such as coronary heart disease [11–14]. However, it is still unclear whether miRNAs can be used as biomarkers of ACS and further evaluate the severity of ACS [15].**

Some MicroRNAs are involved in the pathogenesis of various cardiovascular conditions such as angiogenesis, hypertrophy, heart failure, and fibrosis [7-9]. While others are considered as diagnostic markers for diagnosis of ACS because of their stability in, blood and may serve as targets for revascularization therapy [10].

One of miRNAs, miR-183 which is located at the 7q31-34 locus of human chromosome [11], its expression is tissue specific and highly enriched in visual and sensory organs [12]. Mir-183 has previously been considered to mediate various processes such as cells proliferation, invasion, and apoptosis [13,14]. Mir-183-5p has been studied in various types of cancers, such as human breast cancer [15], esophageal squamous cell carcinoma [16], and gastric cancer [17]. Recently, abnormal expression of circulating miR-183-5p has been detected in coronary syndrome (ACS) patients [18]. However, there are limited studies about the usage of miR-183-5p as a diagnostic marker for AMI.

Mir-208b encoded by β-MHC/Myh7 gene. It belongs to the miRNA-208 family, which plays a vital role in regulating muscle myosin content, myofiber identity, and muscle performance [19, 20]. Mir-208 showed a close association with the development of

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cardiac diseases such as myocardial hypertrophy, cardiac fibrosis, myocardial infarction, arrhythmia, and heart failure [21].

Therefore, the present work was designed to assess of the diagnostic performance of miR-183-5p and miRNA-208b in ACS patients.

SUBJECT AND METHODS

Study Population

This study was conducted on 75 acute coronary syndrome patients attending at cardiology department, faculty of medicine; Ain Shams University in the period from January 2018 till December 2018. Study protocol was approved by the Research and Ethical Committee of faculty of medicine; Ain Shams University. Informed consent was obtained from all participants.

ACS patients were grouped into UA (n =23) and AMI (n = 52) patients. Patients were diagnosed on the basis of assessment of cardiac troponin level, creatine kinase-MB (CK-MB) together with ischemic symptoms, a pathological Q wave, and clinical symptoms within 6 h of chest pain according to American College of Cardiology/American Heart Association (2018 ESC/ACC/AHA/WHF Fourth Universal Definition) guidelines.

In addition to 25- matched sex and age healthy volunteers with normal ECG and no history of CVS disease were enrolled in this study. Subjects with end-stage renal failure, immunological diseases, cardiomyopathy, liver disease, hemorrhagic disorders, radiotherapy or chemotherapy, or inflammatory bowel disease, chronic myopathy and cancer were excluded from the study. All biochemical and clinical data were obtained from data sheet.

Blood samples were collected and centrifuged at 4000 rpm for 20 min. The sera were kept in aliquots and stored at −70 ◦C for molecular analysis.

Methods

Total RNA was purified from the sera samples by miRNEasy extraction kit (Qiagen, Hilden, Germany) according to the kit manual. Concentration & purity of RNA was analyzed by NanoDrop (Thermo Scientific, Waltham, MA, USA) and with Invitrogen™ Qubit™ 3.0 Fluorometer (Thermo Fisher, Waltham, MA, USA). Equal amounts of RNA were used for reverse transcription (RT) using the TaqMan miRNA Reverse Transcription Kit and for amplification by qPCR, using TaqMan MicroRNA Assays of the selected miRNAs, and TaqMan universal master mix (Applied Biosystems, Foster City, CA; Thermo Fisher, Waltham, MA, USA), U6 sn RNA was used as endogenous control for miR-183-5p and miR-208b according to the manufacturer's protocol using Applied Biosystems 7500 FAST Real Time PCR system (Applied Biosystems, Foster City, CA). $2^{-\Delta\Delta Ct}$ method was used for quantitative calculations for miRNAs relative quantification [1].

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Statistical Analyses

Data was statistically analyzed using software package of statistical analysis version 20 (SPSS 20). For quantitative data, Shapiro-Wilk test was performed to determine the type of data distribution, normally distributed data were expressed as mean±SD and non-normally distributed data were expressed as median and interquartile range (25^{th} and 75^{th} percentile). Continuous variables were compared between groups using ANOVA followed by Tukey's post hoc for multiple comparisons or Kruskal-Wallis test followed by Dunn test as a post hoc for multiple comparisons as appropriate. For categorical data, the descriptive measures were presented in frequencies and percentages. Correlation analysis was performed using Spearman and person's correlations as appropriate. To evaluate the diagnostic value of miRNAs, receiver operating characteristic (ROC) curves were constructed. All *p*-values were 2-sided and a *p*-value ≤0.05 was considered significant.

RESULTS

Basic characteristics of study population

Demographic data of the studied groups are listed in (Table 1), there was no significant difference in age, sex, hypertension and diabetes state among the three groups, while unstable angina and AMI patients showed a significant difference in smoking state when compared to the control group (p=0.001 and p<0.001 respectively). In addition, unstable angina and AMI patients who take nitrates exhibited a highly significant difference when compared to the control group (p=0.014 and p=0.013 respectively). Also, unstable angina and AMI patients who take statins showed a significant difference when compared to the control group or to each other (p=0.006, p<0.001, and p=0.021 respectively). Patients with unstable angina and AMI had higher BMI than the controls (p=0.011 and p=0.004 respectively).

Table 2 presented the biochemical and molecular characteristics of the all groups. Compared to the controls, unstable angina and AMI patients showed non-significant difference in levels of total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein-cholesterol. However, levels of triglycerides was increased (p<0.001). In addition, serum CK-MB, and troponin I were significantly elevated in unstable angina patients (p=0.004 and p=0.001, respectively) .In the same context, AMI patients exhibited a significant increase in levels of CK-MB, and troponin I (p<0.001). Whereas in comparison to unstable angina patients, AMI patients showed a significant increase in levels of CK-MB, and troponin I (p=0.034, p=0.009 respectively).

Regarding miR-183-5p expression levels, miR183-5p was significantly upregulated in serum of unstable angina and AMI patients compared to normal controls (p<0.001). In contrast, there was no significant differences in miR-183-5p expression levels between unstable angina and AMI patients (p=0.230).

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On the other hand, miR -208b was significantly over-expressed in serum of AMI patients compared to unstable angina patients and normal controls ($p<0.001$). In addition, miR-208b expression levels was over-expressed in unstable angina patients compared to the control group ($p=0.044$).

Correlation of miR-183-5p expression levels with the studied parameters

Mir-183-5p expression levels showed a significant negative correlation with CK-MB and troponin I ($p=0.048$, and $p=0.008$, respectively) in unstable angina patients. While a significant positive correlation with miR-208b was found in AMI patients ($p=0.001$). However, there were no correlations with any variables in the control group (Table 3).

Correlation of miR-208b expression levels with the biochemical parameters

Regarding AMI group, miR-208b expression level was positively correlated with CK-MB ($p=0.029$).On the other, there were non-significant correlations between miR-208b expression levels and any variables in patients with unstable angina (Table 4).

Efficacy of miR-183-5p and miR -208b as potential diagnostic biomarkers for AMI

Figure (1a) demonstrates the ROC curves of troponin I levels to discriminate AMI patients from the control and unstable angina groups. The figure showed that troponin I had diagnostic value for AMI with an area under curve (AUC) of 0.872 (95% confidence interval [CI]: 0.799–0.945, $p < 0.001$) at an optimal cutoff point of ≥11.5 ng/ml which could yield 86% sensitivity and 77% specificity. Whereas, Figure (1b) showed that miR-183-5p had the highest diagnostic value with an AUC of 0.843 (95% CI: 0.759– 0.926, $p < 0.001$) at a cutoff point of ≥8.03, and with a sensitivity and specificity of (92% and 73%). While, Figure (1c) showed that miR- 208b had diagnostic value with an AUC of 0.880 (95% CI: 0.817– 0.943, $p < 0.001$) at a cut-off point of ≥2.09, which is associated with a sensitivity of 96% and specificity of 60%.

DISCUSSION

Acute coronary syndrome is a rapidly progressive cardiovascular disease with high mortality rate. The occurrence of ACS is currently assessed clinically through risk score algorithms, myocardial enzyme, angiography and electrocardiography [22]. However, they all have different drawbacks. For example, myocardial enzymes in patients with ACS are not diagnostic of unstable angina [23]. Angiography is an invasive test with a risk of complications and does not easily screen a wide range of patients [24] The ECG is an important diagnostic tool in acute coronary syndrome; however, it lacks sensitivity and about 30–50% of patients may initially present symptoms with normal ECG [25]. Thus, there is an urgent need to identify new diagnostic and therapeutic biomarkers of ACS; so this study aimed to assess the diagnostic value of miR183-5p and miR-208b. Micro-RNAs are involved in endothelial dysfunction, inflammation, apoptosis, angiogenesis, atherosclerosis, and some pathological processes involved in cardiovascular diseases [26], and some miRNAs of circulatory system may be potential biomarkers of these diseases [27].miR-135a, miR-31, miR-378, and miR-147 are known to be biological

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markers of stable coronary heart disease [28]; Other micro- RNAs like miR-1, miR-126, and miR-133a have potential value in the diagnosis of UA [29]; also miR-208b, miR-499, and miR-1 play a key role in the diagnosis, progression, and prognosis of acute myocardial infarction (AMI) [30]. However, it is still unclear whether miRNAs are differentially expressed with changes in the severity of coronary heart disease, including coronary stenosis and myocardial damage.

This study showed that miR183-5p and miR-208b were significantly up-regulated in serum of adult unstable angina and AMI patients compared to normal controls. While, there was non-significant difference between unstable angina and AMI patients in miR-183-5p expression levels. But, miR-208b was significantly overexpressed in AMI patients compared to unstable angina patients.

The abnormal expression of miR-183-5p has been widely reported in several human diseases [31, 32, 33]. Nevertheless, limited studies discussed the expression levels of miR-183 in ACS patients. Bin et al. reported that miR-183-5p was involved in atherogenesis, where it was highly expressed in atherosclerotic patients and had the ability to be used as a biomarker for atherosclerosis [18]. Moreover, Tong et al. noticed that plasma miR-183-5p could potentially serve as an early marker for young ACS patients without malignancy, which is supported with the results of this study [34]. In addition Xingxing et al., found that patients with MI had the highest expression level of exosomal miR-183 in comparison with stable angina patients and healthy controls where this may be due to that miR-183-5p was involved in cardiomyocyte adrenergic signaling *via* the regulation of certain protein kinases [35].

Regarding miR-208b, Liu et al. showed that serum miR-208b was significantly elevated in MI patients and myocardial tissues of MI mice [36]. Further, Li et al. found that miR-208 were significantly upregulated in AMI and angina pectoris patients compared to healthy control subjects which in consistent with our results [37]. The high expression level of miR-208b may be explained *via* that following myocardial damage, cardio-enriched miRNAs are released into the bloodstream from necrotic cardiomyocytes, which subsequently have a paracrine effect on the heart [38]. In addition, miR-208b targets several mRNAs that were translated into proteins that involved in the generation of cardiac excitation and propagation, as well as other protein involved in RNA translocation and cardiac hypertrophic response [39,40].

CONCLUSIONS

This study indicated that miR-183-5p and miR-208b were over-expressed in sera unstable angina and AMI patients. The mechanism of this up-regulation is not fully understood and needs further clarification. In addition, according to the ROC analysis, the serum levels of miR-183-5p had the highest diagnostic to discriminate adult AMI patients.

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Institutional Review Board Statement: The study was approved by the Ain Shams Research Ethics Committee, Faculty of Medicine, Egypt, and in accordance to the guidelines of the Declaration of Helsinki.

Informed Consent Statement: Informed consents were taken from all participants involved in the study.

Data Availability Statement: The data reported in this study are available on request from the corresponding authors.

Conflicts of Interest: All the authors have no conflict of interest to disclose

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Table 1: Basic characteristics of the studied groups

	Control (n=25)	Unstable angina (n=23)	AMI (n=52)	*p*-value
Age (years)	54±9.6	59.9±12.9	54.7±10.4	0.113
Gender (male/female), n (%)	20/5 (80/20)	18/5 (78.3/21.7)	42/10 (80.8/19.2)	0.969
BMI(Kg/m^2)	25±1.7	27.5±4.2[a]	27.3±2.8[a]	0.003
Smoking, n (%)				<0.001
Positive Negative	6(24) 19(76)	17(73.9) 6(26.1)	37(71.2) 15(28.8)	
Hypertension, n (%)				0.362
Positive Negative	8(32) 17(68)	5(21.7) 18(78.3)	20(38.5) 32(61.5)	
Diabetes, n (%)				0.104
Positive Negative	10(40) 15(60)	4(17.4) 19(82.6)	22(42.3) 30(57.7)	
Medications				
β-Blockers, n (%)				0.686
Positive Negative	6(24) 19(76)	8(34.8) 15(65.2)	14(26.9) 38(73.1)	
ACE I, n (%)				0.266
Positive Negative	5(20) 20(80)	2(8.7) 21(91.3)	13(25) 39(75)	
Nitrates, n (%)				0.042
Positive Negative	0(0) 25(100)	5(21.7) 18(78.3)	11(21.2) 41(78.8)	
Statins, n (%)				<0.001
Positive Negative	1(4) 24(96)	8(34.8) 15(65.2)	33(63.5) 19(36.5)	

Data are expressed as mean ± SD for Gaussian variables, and frequencies (percentages) for categorical variables. AMI: acute myocardial infarction; BMI: body mass index; ACEI: angiotensin converting enzyme inhibitors

Table 2: Biochemical and molecular variables among the studied groups

	Control (n=25)	Unstable angina (23)	AMI (n=52)	*p*-value
TC (mg/dl)	200±30.6	210.2±27.9	212.2±34.9	0.295
TG (mg/dl)	68.4±27.7	120±57.9[a]	133±41.8[a]	<0..001
HDL-C (mg/dl)	30(25-43)	29(27-40)	30(27.25-35)	0.822
LDL-C (mg/dl)	118.2±23.4	132.4±25.2	126.1±31.4	0.219
CK-MB (IU/l)	8(2.5-13)	23(11-34)[a]	33.5(24-55.75)[a,b]	<0.001
Troponin I(ng/ml)	0.2(0.1-0.4)	11(0.7-33)[a]	56(29-89.75)[a,b]	<0.001
miR-183-5p relative expression	0.23(0.08-0.88)	9.46(7.54-12.09)[a]	12.24(10.35-15.84)[a]	<0.001
miR-208b relative expression	0.4±0.26	5.5±2.6[a]	12.9±9.8[a,b]	<0.001

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Gaussian variables are expressed as mean ±SD and non- Gaussian variables as median (inter-quartile range). TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein-cholesterol

Table 3: Correlation analysis of the studied parameters with miR-183-5p relative expression

	Control (n=25)		**Unstable angina (n=23)**		**AMI (n=52)**	
	r	***p*-value**	r	***p*-value**	r	***p*-value**
Age (years)	0.05	0.831	0.01	0.957	-0.02	0.868
BMI(Kg/m^2)	-0.02	0.909	-0.14	0.524	-0.03	0.836
TC (mg/dl)	-0.17	0.410	0.31	0.139	-0.04	0.778
TG (mg/dl)	-0.20	0.342	-0.54	0.007	0.18	0.199
HDL-C (mg/dl)	0.10	0.633	0.11	0.633	-0.06	0.698
LDL-C (mg/dl)	-0.45	0.026	0.10	0.646	-0.06	0.684
CK-MB (IU/l)	0.05	0.811	-0.42	0.048	0.19	0.174
Troponin I(ng/ml)	-0.003	0.987	-0.58	0.004	0.18	0.215
miR-208b	0.124	0.554	0.064	0.770	0.465	0.001

BMI: body mass index; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein-cholesterol

Table 4: Correlation analysis of the studied parameters with miR-208b relative expression

	Control (n=25)		**Unstable angina (n=23)**		**AMI (n=52)**	
	r	***p*-value**	r	***p*-value**	r	***p*-value**
Age (years)	-0.21	0.312	-0.18	0.396	-0.09	0.517
BMI(Kg/m^2)	0.22	0.300	-0.30	0.168	-0.22	0.120
TC (mg/dl)	-0.12	0.570	-0.07	0.741	-0.12	0.380
TG (mg/dl)	0.31	0.137	-0.32	0.139	-0.02	0.897
HDL-C (mg/dl)	-0.09	0.677	0.16	0.463	-0.12	0.417
LDL-C (mg/dl)	-0.02	0.913	-0.17	0.451	0.003	0.985
CK-MB (IU/l)	-0.08	0.702	0.20	0.358	0.30	0.029
Troponin I(ng/ml)	-0.22	0.282	0.10	0.664	0.21	0.143

BMI: body mass index; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein-cholesterol

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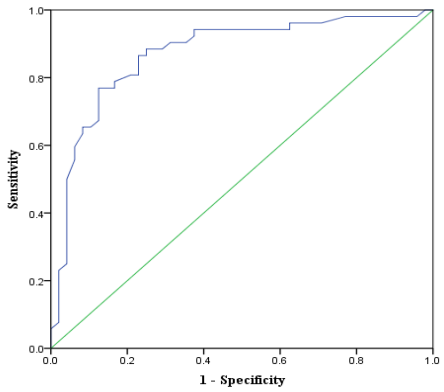
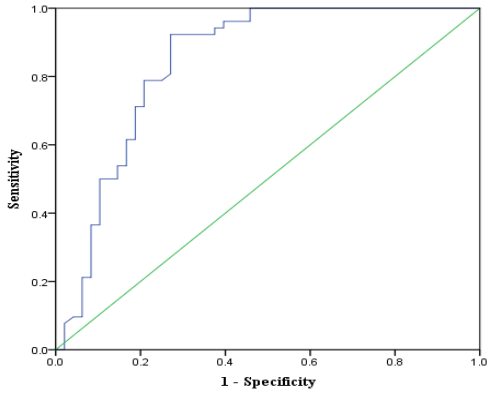
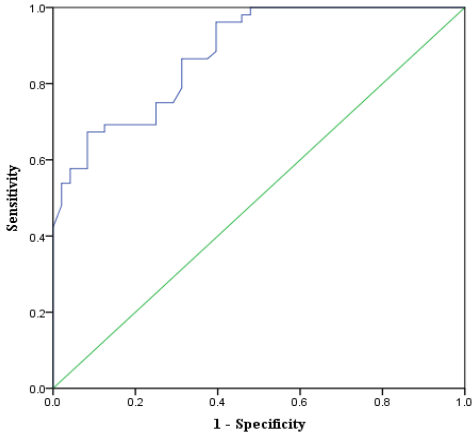
a)



b)



c)



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	AUC	95%CI		*p*-value	Cut-off ≥	Sensitivity (%)	Specificity (%)
		Lower bound	Upper bound				
Troponin I (ng/ml)	0.872	0.799	0.945	<0.001	11.5	86%	77%
miR-183-5p relative expression	0.843	0.759	0.926	<0.001	8.03	92%	73%
miR-208b relative expression	0.880	0.817	0.943	<0.001	2.09	96%	60%

Fig (1): ROC curves of a) Troponin I concentration, b) miR-183-5p relative expression, and c) miR-208 relative expression for discriminating AMI patients AUC: area under curve, CI: Confidence interval