Journal of Medicinal and Chemical Sciences 7 (2024) 59-77

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Review Article

Implication of Plasma miR 122 and miR 151-3p Levels in Diagnosis and Prognosis of Acute Coronary Syndrome Patients

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ARTICLE INFO

Article history

Receive: 2023-07-19
Received in revised: 2023-08-26
Accepted: 2023-09-10
Manuscript ID: JMCS-2308-2246
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatima Ramezani
Editor who approved publication:
Dr. Kiran Chinthapally

DOI:10.26655/JMCHEMSCI.2024.1.7

KEYWORDS

ACS
miR-122
miR-151-3p
NSTEMI
RT-Qpcr
STEMI

ABSTRACT

Background: Acute coronary syndrome (ACS) has a significant morbidity and fatality rate. An early diagnosis and accurate prognosis are necessary. Therefore, biomarkers that are associated with coronary artery stenosis and hazard for coronary artery disease (CAD) development are far required. The objective of the current study is to identify the role of plasma miR-122 and miR 151-3p as diagnostic and prognostic indicators in patients with ACS.
Methods: A total of 100 Egyptian subjects were enrolled that included 25 unstable angina (UA) patients, 25 NSTEMI patients, 25 STEMI patients, and 25 healthy volunteers. Quantitative real-time PCR (RT-PCR) for both plasma miR-122 and 151-3p expression were performed and Cardiac Catheterization had been performed for displaying the severity of stenosis.
Results: There was steadily significant elevation in miRNA-122 and miR151-3p levels from control group to STEMI group ($p<0.001$). In studied cases, there were substantial positive correlation between both miR-122 and miR 151-3p with cardiac Troponin ($p<0.001$) and coronary stenosis ($p<0.001$).
Conclusion: Both miR-122 and miR-151 -3p were diagnostic biomarkers for ACS and were capable of evaluation of the grade of coronary artery stenosis to expect the necessity for percutaneous coronary intervention (PCI) in medical practice. Moreover, they have prognostic value for adverse cardiac events (ACE).

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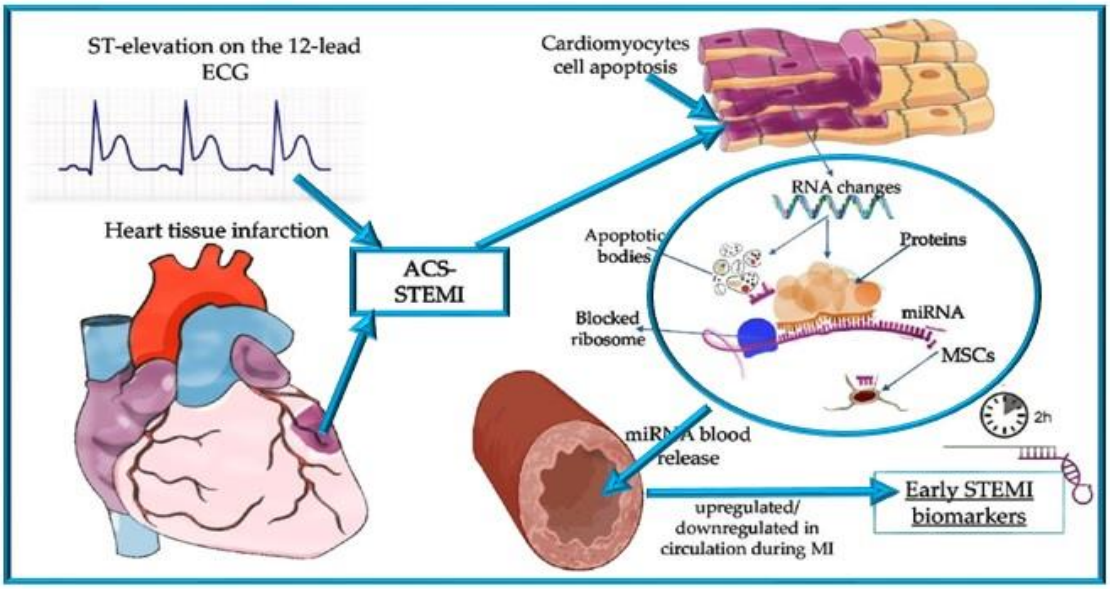
GRAPHICAL ABSTRACT



Introduction

In spite of continuous progress achieved in the identification and management of acute coronary syndromes, it remains the chief mortality cause, with approximately 50% of these deaths owing to ischemic heart disease [1, 2] and imposes a heavy medical burden on society. About 12% of disability-adjusted life-years lost every year are caused by ischemic heart disease [3].

Acute myocardial infarction (AMI), unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI), and other ischemic disorders are all included in acute coronary syndromes [4]. Even though high sensitivity troponin is the golden typical cardiac definite biomarker [5], the main limitation is its incapability to detect UA patients [6] and its truncated specificity in distinguishing ACS cases from other clinical conditions, such as acute myocarditis, heart failure without CAD, and pulmonic embolism [7]. Consequently, there is a definitive necessity to find new sensitive indicators to precisely diagnose acute coronary syndromes cases and contribute in their treatment strategy.

MicroRNAs (miRNAs) are minor non-coding molecules which has a significant role in atherosclerotic progress and their levels changed in CAD events [8, 9]. In 2008, Lawrie *et al.* suggested the existence of miRNAs in body fluids, which prompted an increase in investigation of circulatory miRNAs as indicator of different human diseases comprising CAD in the subsequent years [10, 11]. Numerous studies proved that circulatory miRNAs are appropriate indicators owing to their specificity besides easy accessibility in biological fluids [12]. Formerly, numerous studies revealed that circulatory miRNA appear to be hopeful indicator for the CAD identification [13]. Though, restricted evidence has been showed concerning the correlation of miRNAs with CAD prospects and the prognostic capability compared to prior CAD biomarkers such as high-sensitivity troponin [14]. Hence, whether circulatory miRNAs can aid as an operational prognostic indicator for CAD events in overall people is still unidentified MiR-122 possesses variable and even contrasting guiding outcomes on the cardiovascular system and fixes its target genes to regulate the values of pro-inflammatory factors [15].

Remarkably, miR-122 acts as a hazard indicator of cardiovascular fibrosis and seems to be a main contributor in the progress of CVD, comprising hypertension, HF, atherosclerosis, and myocardial infarction (MI). Suppression of miR-122 by anti-miRNA stratagems triggered a decrease in plasma cholesterol values [16]. Thus, this research targets to identify the role of miRNA 122 in diagnosis and expecting the ACS prognosis.

Numerous studies had observed a relationship of miR-151-3p with cancer such as liver cancer, cancer breast, and stomach cancer [17, 18]. The metastasis stimulating element miR-151 was recognized in the chromosomal variant region of hepatic cancer. The intensification of chromosome 8q24.3 was found to cause the increased expression of miR-151 in HCC, besides augmented the metastasis of HCC cells [19]. Liu *et al.* revealed that miR-151-3p negatively regulates native immune reactions and inflammation by impeding IL-6 expression by decreasing the expression the Stat3 [20]. However, the title role of miR 151-3p in ACS diagnosis is not clear up till now. Numerous studies had observed a relationship of miR-151-3p with cancer such as liver cancer, cancer breast, and stomach cancer [17, 18]. The metastasis stimulating element miR-151 was recognized in the chromosomal variant region of hepatic cancer. The intensification of chromosome 8q24.3 was found to cause the increased expression of miR-151 in HCC, besides augmented the metastasis of HCC cells [19]. Liu *et al.* revealed that miR-151-3p negatively regulates native immune reactions and inflammation by impeding IL-6 expression by decreasing the expression the Stat3 [20]. However, the title role of miR 151-3p in ACS diagnosis is not clear up till now. The objective of the current study is to profile and validate the potential diagnostic value of the combination of plasma miRNA 122 and miRNA 151-3p levels in ACS patients, studying the correlation between miR-122 and miR-151 values and the severity of coronary artery stenosis, and evaluation of their prognosis via studying their correlation with adverse cardiac complications in ACS patients.

Materials and Methods

Research subjects

This study is a case-control study. It was performed at Medical Biochemistry Department and Cardiology Department at Zagazig Faculty of Medicine, Egypt in the period from January 2023 to July 2023. Seventy five (75) ACS Egyptian patients were involved in the study. They were allocated equally into 3 groups: UA patients, NSTEMI patients, and STEMI patients. UA patients presented with typical chest pain, the ECG not showing ST- segment elevations or new left bundle branch block, cardiac troponin negative. NSTEMI patients were diagnosed by the ischemic cardiac pain, no ST segment raise, elevated cardiac troponin. STEMI patients were identified by chest pain, new or presumed new ST segment elevation, and elevated cardiac troponin. The control group included 25 healthy Egyptian age and gender-matched participants. The coronary angiography done later documented coronary artery disease (CAD) in all ACS cases and assessed the severity of coronary stenosis which was recorded and tabulated. The patients were observed for the occurrence of ACE (adverse cardiac events e.g., heart failure or arrhythmia). A written approval was signed by all participants proceeding to the research. The research was applied in agreement with the Declaration of Helsinki. The study was accepted by Institutional Research Board Committee of Zagazig Faculty of Medicine. The following was administered to each participant: ECG was performed to detect ischemic changes after a detailed history taking that included age, sex, history of smoking, family history, hypertension, and diabetes. We tested and documented the levels of fasting blood sugar, lipid profile, and cardiac troponin (cTn) which was assessed and correlated later with miRNA level. The coronary angiography done far ahead documented coronary artery disease (CAD) in all ACS cases and assessed the severity of coronary stenosis which was recorded and tabulated.

Research method

Collection of venous blood samples

3 ml of venous blood samples were withdrawn immediately on admission but before

angiography; via antecubital venous puncture. Blood samples were withdrawn on EDTA for real-time PCR analysis of plasma miRNA-122 and miRNA 151-3p levels. miRNA extraction from plasma was performed by miRNeasy kits from Qiagen, Germany, catalogue No RY43. All steps were performed in an environment free of RNA contamination.

Synthesis of cDNA

Then miRNA was reverse transcribed via miScript IIRT kit Qiagen, Germany catalogue no: 218161. The cDNA was transferred to a -20 °C freezer.

Real time amplification for miRNA levels

The augmentation was accomplished in a 20 µL combination comprising 5µL of the cDNA, 100 pmol/mL of every primer miRNA-122, miRNA 151-3p, or RNU6 as internal control, 10 µL 2x QuantiiTect SYBER Green PCR Master Mix (Qiagen) and 4 µL distilled water. The augmentation was achieved by Real time Cycler (Strategene Mx3005P) consistent with the next strategy; 95 °C for 15 min first motivation step before 40 cycle of 95 °C for 15 sec, 55 °C for 30 sec and finally 70 °C for 30 sec. The fold changes of the miRNA expression detected in patients in comparison to healthy group was evaluated by the 2-ΔΔCt method.

Results and Discussion

Demographic properties among the studied groups are presented in Table 1. A total of 100 individuals were evenly divided into four groups: controls (25 patients, 39.88±6 years, 56% males vs. 44% females), UA patients (25 patients, 41 ± 6.2 years, 52% males vs. 48% females), NSTEMI patients (25 patients, 40.88±5.9 years, 56% males vs. 44% females) and STEMI patients (25 patients, 40.5±6.6 years, 56% males vs. 44% females). There were significant differences between the studied groups (especially between controls and other patients groups) regarding risk factors as BMI, DM, HTN, hyperlipidemia, and family history, as illustrated in Table 1.

The mean cardiac troponin level in controls was 3.51±0.79 pg /mL, while it was 5.44±2.65 pg/mL in UA group, 55.07±45.52 pg/mL in NSTEMI group, and 190.34±116.08 pg/mL in STEMI group. It was found that there was statistically substantial rise of cardiac troponin in NSTEMI and STEMI groups compared to controls. Also, significant elevation of cardiac troponin NSTEMI and STEMI groups was found in comparison to UA group and between STEMI and NSTEMI group, as provided in Table 2.

The mean coronary artery stenosis severity in UA group was 57.6±9.03%, while it was 73.6± 8.10 % in NSTEMI group and 91.04±6.28% in STEMI group. It was found that there was statistically significant elevation of coronary artery stenosis severity in NSTEMI and STEMI groups related to UA group. Also, significant elevation of coronary artery stenosis severity in STEMI group in comparison to NSTEMI group, as illustrated in Table 3.

The mean miRNA-122 level in controls was 0.99± 0.08, while it was 1.32±0.09 in UA group, 1.58±0.15 in NSTEMI group, and 1.90±0.13 in STEMI group. There was steadily significant elevation in miRNA-122 level from control group to STEMI group, as demonstrated in Table 4 and Figure 1. The mean miRNA-151-3p level in controls was 1.02±0.08, while it was 1.29±0.11 in UA group, 1.54±0.12 in NSTEMI group, and 1.92±0.11 in STEMI group. There was steadily significant elevation in miRNA-151-3p level from control group to STEMI group as illustrated in Table 4.

Regarding adverse cardiac events, seven cases (28%) in UA group, 13 (52%) in NSTEMI group, and 20 (80%) cases in STEMI group reported adverse cardiac events (ACE). It was found that there was statistically significant higher incidence of ACE in NSTEMI, STEMI, and UA groups compared to control group.

Also, significant higher incidence of ACE in STEMI group compared to UA group, as illustrated in Table 5. The mean miRNA 122 level in cases with ACE was 1.70±0.23 and in cases without ACE, it was 1.28±0.32. There was significant elevation in miRNA 122 level in cases with ACE compared to cases without ACE, as presented in Table 5. The mean mi-RNA 151-3p level in cases with ACE was 1.65±0.29 and in cases without ACE was 1.30±0.31.

Table 1: Demographic characteristics among the studied groups

		Control group (n.= 25)		UA group (n.= 25)		NSTEMI group (n.= 25)		STEMI group (n.= 25)		Test value	P-value						
		No.	%	No.	%	No.	%	No.	%			P1	P2	P3	P4	P5	P6
Gender	Female	11	44.0%	12	48.0%	11	44.0%	11	44.0%	X^2 = 2.74	0.434 (NS)	1.00	0.396	0.256	0.570	0.393	1.00
	Male	14	56.0%	13	52.0%	14	56.0%	14	56.0%								
Age (years)	Mean± SD	39.88± 6		41 ± 6.2		40.88± 5.9		40.5± 6.6		F= 8.89	0.523	0.825	0.765	0.512	0.516	0.915	0.886
	Range	30.0-53.0		30.0-53.0		30.0-53.0		30.0-53.0									
BMI (Kg/m2)	Median	23.6 (22.8-24.6)		29.6 (28.7-31.2)		30.7 (27.7-33.8)		32.9 (28.7-34.2)		KW = 51.4	<0.001 (HS)	<0.001	<0.001	<0.001	0.733	0.167	0.298
	Range	22.7-24.8		26.9-37.6		23.8-38.7		24.5-42.9									
Smoking	Negative	25	100.0%	20	80.0%	16	64.0%	14	56.0%	X^2 = 15.09	0.002 (HS)	0.059	0.003	0.001	0.345	0.130	0.773
	Positive	0	0.0%	5	20.0%	9	36.0%	11	44.0%								
DM	Negative	25	100.0%	13	52.0%	9	36.0%	3	12.0%	X^2 = 41.44	<0.001 (HS)	<0.001	0.003	<0.001	0.393	0.006	0.098
	Positive	0	0.0%	12	48.0%	16	64.0%	22	88.0%								
HTN	Negative	25	100.0%	12	48.0%	8	32.0%	7	28.0%	X^2 = 33.0	<0.001 (HS)	<0.001	<0.001	<0.001	0.386	0.244	1.00
	Positive	0	0.0%	13	52.0%	17	68.0%	18	72.0%								
hyperlipidemia	Negative	25	100.0%	16	64.0%	9	36.0%	9	36.0%	X^2 = 28.57	<0.001 (HS)	0.003	<0.001	<0.001	0.090	0.090	1.00
	Positive	0	0.0%	9	36.0%	16	64.0%	16	64.0%								
Family history	Negative	25	100.0%	20	80.0%	15	60.0%	5	20.0%	X^2 = 38.46	<0.001 (HS)	0.059	0.001	<0.001	0.217	<0.001	0.009
	Positive	0	0.0%	5	20.0%	10	40.0%	20	80.0%								

P1: Comparison between control and UA group, P2: Comparison between control and NSTEMI group P3: Comparison between control and STEMI group, P4: Comparison between UA group and NSTEMI group, P5: Comparison between UA and STEMI group, and P6: Comparison between NSTEMI group and STEMI group.

Table 2: Cardiac Troponin cTn (pg /mL) among the studied groups

		Control group (n.= 25)	UA group (n.= 25)	NSTEMI group (n.= 25)	STEMI group (n.= 25)	Test value	P-value	P1	P2	P3	P4	P5	P6
Cardiac Troponin "cTn" (pg/mL)	Mean ± SD	3.51± 0.79	5.44± 2.65	55.07± 45.52	190.34± 116.08	KW = 59.4	<0.001 (HS)	0.220	<0.001	<0.001	0.012	<0.001	0.001
	Median	3.65 (2.98-4.07)	5.26 (3.15-7.71)	53.4 (3.67-94.6)	175.6 (75.45-270.4)								
	Range	1.94-4.64	2.15-9.98	2.64-133.2	33.14-394.6								

p≤0.05 is considered statistically significant, p≤0.01 is considered highly statistically significant, SD: standard deviation, analysis done by KW: Kruskal-Wallis Test. P1: Comparison between control and UA group, P2: Comparison between control and NSTEMI group P3: Comparison between control and STEMI group, P4: Comparison between UA group and NSTEMI group, P5: Comparison between UA and STEMI group, and P6: Comparison between NSTEMI group and STEMI group.

Table 3. Coronary artery stenosis severity among the studied groups

	UA group (n.= 25)	NSTEMI group (n.= 25)	STEMI group (n.= 25)	Test value	P-value	P1	P2	P3
Coronary Artery Stenosis Severity (%)	Mean± SD	57.6± 9.03	73.6± 8.10	KW= 58.1	<0.001 (HS)	<0.001	<0.001	<0.001
	Median	60.0 (50.0- 60.0)	75.0 (70.0- 80.0)					
	Range	30.0- 70.0	60.0- 85.0					

Table 4: The miRNA-122 and miRNA 151-3p levels among the studied groups

		Control group (n.= 25)	UA group (n.= 25)	NSTEMI group (n.= 25)	STEMI group (n.= 25)	Test value	P-value	P1	P2	P3	P4	P5	P6
miRNA-122 level	Mean ± SD	0.99± 0.08	1.32± 0.09	1.58± 0.15	1.90± 0.13	KW= 85.5	<0.001 (HS)	0.001	<0.001	<0.001	0.008	<0.001	0.004
	Median	0.97 (0.95-1.02)	1.31 (1.3-1.34)	1.62 (1.58-1.64)	1.95 (1.90-1.97)								
MiRNA 151-3p level	Mean ± SD	1.02± 0.08	1.29± 0.11	1.54± 0.12	1.92± 0.11	KW= 89.58	<0.001 (HS)	0.002	<0.001	<0.001	0.004	<0.001	0.002
	Median	1.02 (0.99-1.05)	1.30 (1.28-1.31)	1.60 (1.40-1.60)	1.94 (1.90-1.96)								

P1: Comparison between control and UA group, P2: Comparison between control and NSTEMI group P3: Comparison between control and STEMI group, P4: Comparison between UA group and NSTEMI group, P5: Comparison between UA and STEMI group, and P6: Comparison between NSTEMI group and STEMI group

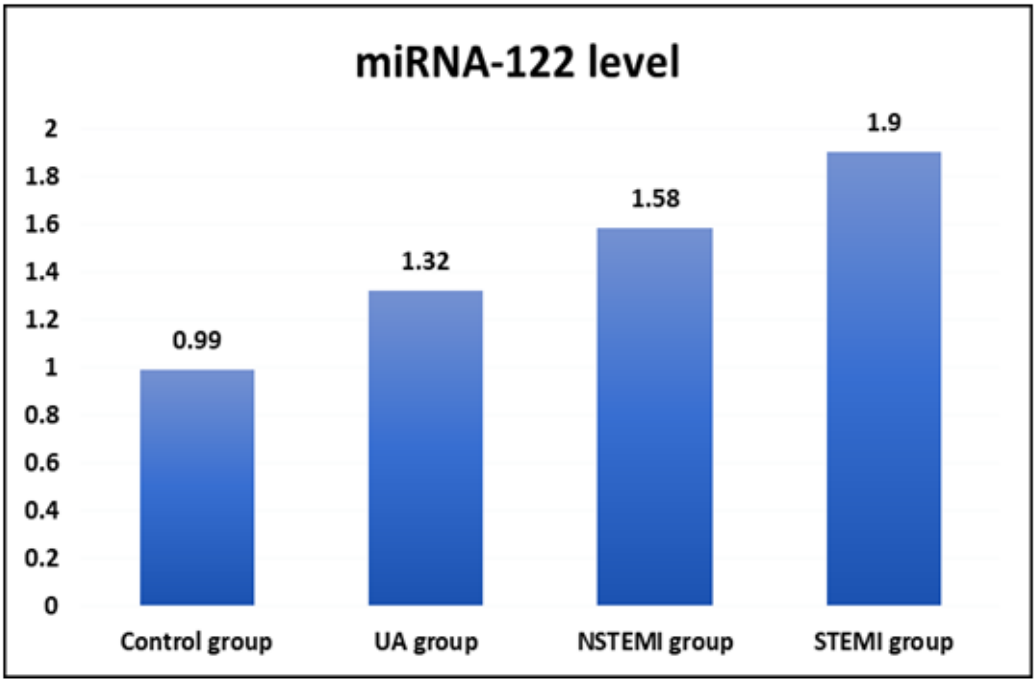


Figure 1: Comparison between the study groups regarding miRNA-122 level

Table 5: Correlation between mi-RNA 151, miRNA-122, and adverse cardiac events (ACE)

		MiRNA 151 -3p			Mann-Whitney U test		MiRNA 122		Mann-Whitney test	
		Mean	SD	Median	Test value	P-value	Mean	SD	Test value	P-value
ACE	No	1.30	0.31	1.29	4.906	<0.001 (HS)	1.28	0.32	5.836	<.001(HS)
	Yes	1.65	0.29	1.60			1.7	0.23		

There was significant elevation in miRNA-151-3p level in cases with ACE compared to cases without Adverse cardiac events (ACE), as listed in Table 5.

In studied patients, there was significant positive correlation between miRNA-122 with cardiac Troponin and coronary stenosis (r=0.785), as illustrated in Table 6 and Figures 2 and 3. Also, there was considerable positive correlation between miRNA 151-3p with cardiac Troponin (r=0.708) and coronary stenosis (r=0.820), as illustrated in Table 6 and Figures 2 and 3.

The ROC analysis confirmed a good power of miRNA-122 to detect coronary stenosis in patients at cut off 1.52 with sensitivity and specificity of 80% and 95%, respectively, as illustrated in Table 7 and Figure 4. Also, miRNA-151-3p showed a good power to detect coronary stenosis in patients at cut off 1.56 with sensitivity and specificity of 82.9% and 90%, respectively, as illustrated in Table 7 and Figure 4. Combination of both miRNA-122 and miRNA-151-3p increases the power with sensitivity and specificity of 85.7% and 92.5%, correspondingly, as illustrated in Table 7 and Figure 4.

The ROC analysis confirmed a good power of miRNA-122 to distinguish patients from the control group at cut off 1.17 with sensitivity and specificity of 100% and 97.3%, correspondingly, as illustrated in Table 8 and Figure 5. Also, miRNA-151-3p showed a good power to distinguish patients from the control group at cut off 1.15 with sensitivity and specificity of 100% and 94.7% individually, as demonstrated in Table 8 and Figure 5. Combination of both miRNA-122 and miRNA-151-3p increases the power with sensitivity and specificity of 100% and 100%, correspondingly, as shown in Table 8 and Figure 5.

The ROC analysis confirmed a good power of miRNA-122 to distinguish patients with ACE from patients without ACE at cut off 1.4 with sensitivity and specificity of 85% and 75%, correspondingly, as illustrated in Table 9 and Figure 6.

Table 6: Correlation between mi-RNA-122, miRNA 151-3p with cardiac Troponin and coronary stenosis in patients groups

	MiRNA-122		MiRNA 151-3p	
	r	p- value	r	p-value
Troponin	0.724	<0.001	0.708	<0.001
coronary stenosis	0.785	<0.001	0.820	<0.001

r: Spearman rho

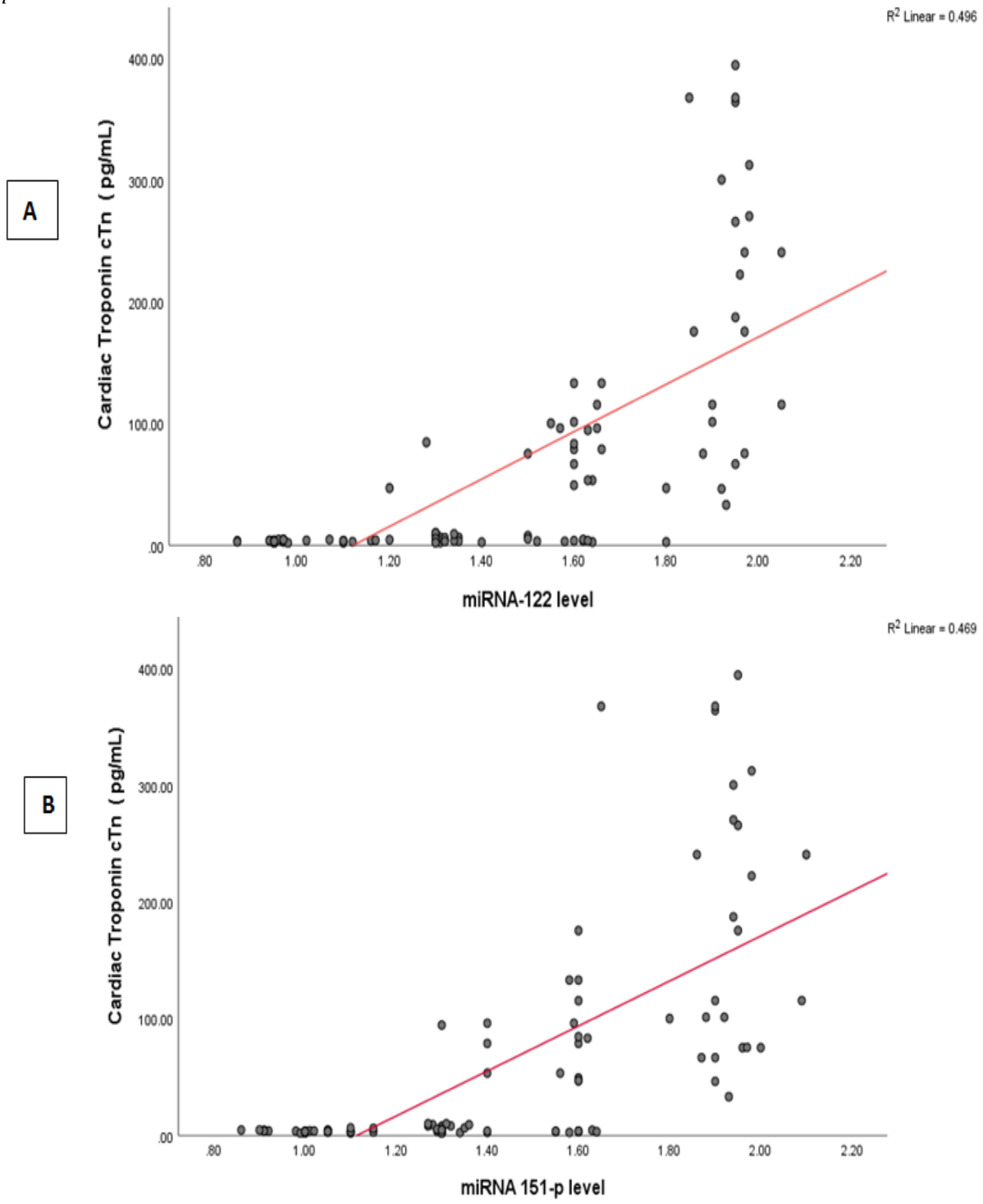


Figure 2: Scatter-plot showing significant positive correlation between cardiac Troponin and miRNA-122 in (A) and miRNA 151-3p in (B)

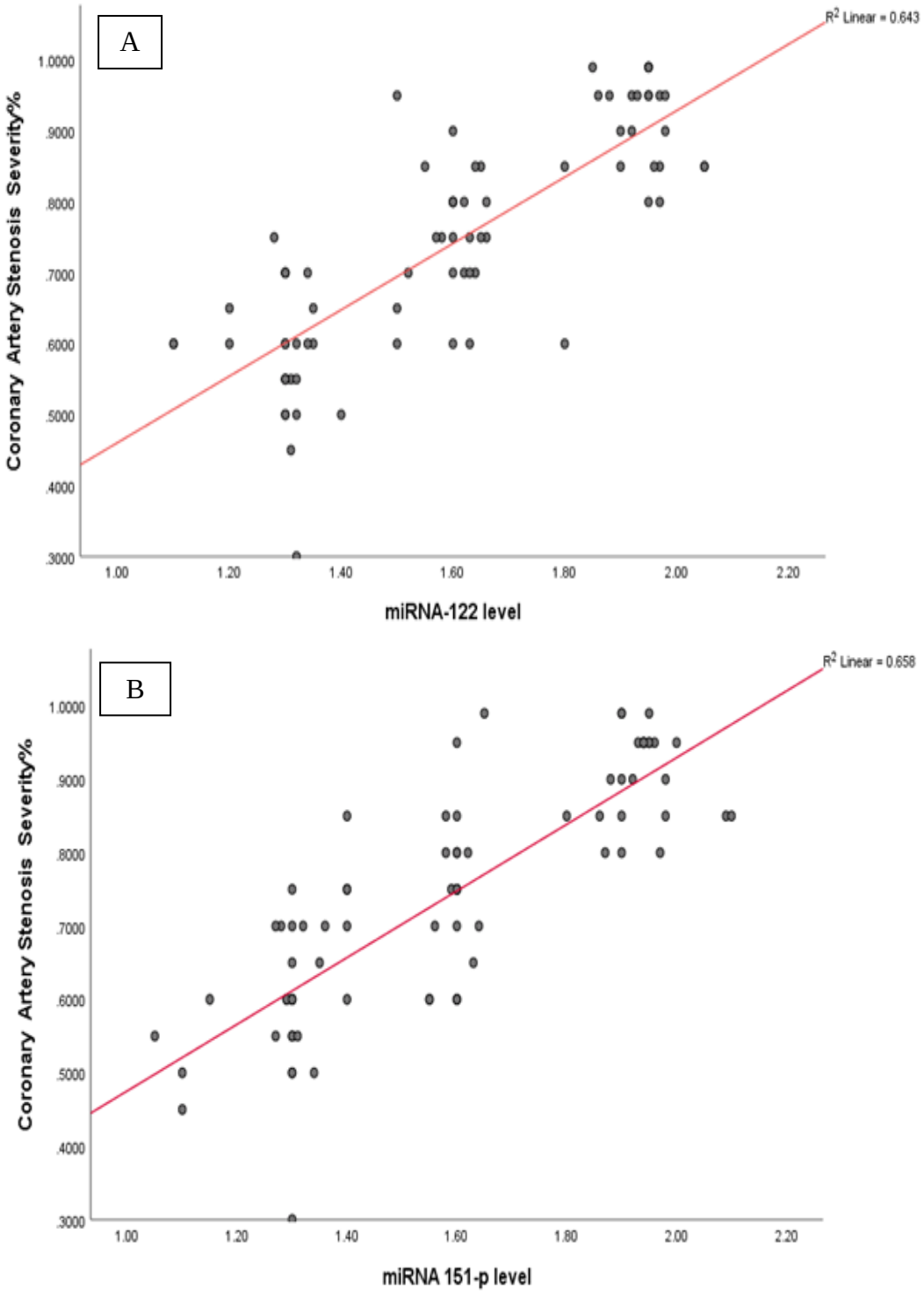


Figure 3: Scatter-plot showing significant positive correlation between coronary artery stenosis and miRNA-122 in (A) and miRNA 151-3p in (B)

Table 7: Area under the curves, optimal cut-off points, and validity of miRNA 122, miRNA 151-3p, and combined miRNA 122+miRNA 151-3p to detect coronary artery stenosis in patients

Parameter	Cutoff value	AUC	Sensitivity	Specificity	P value
miRNA 122	>1.52	0.925	80%	95%	<0.001
MiRNA 151-3p	>1.56	0.929	82.9%	90%	<0.001
miRNA 122 + miRNA 151-3p		0.964	85.7%	92.5%	<0.001

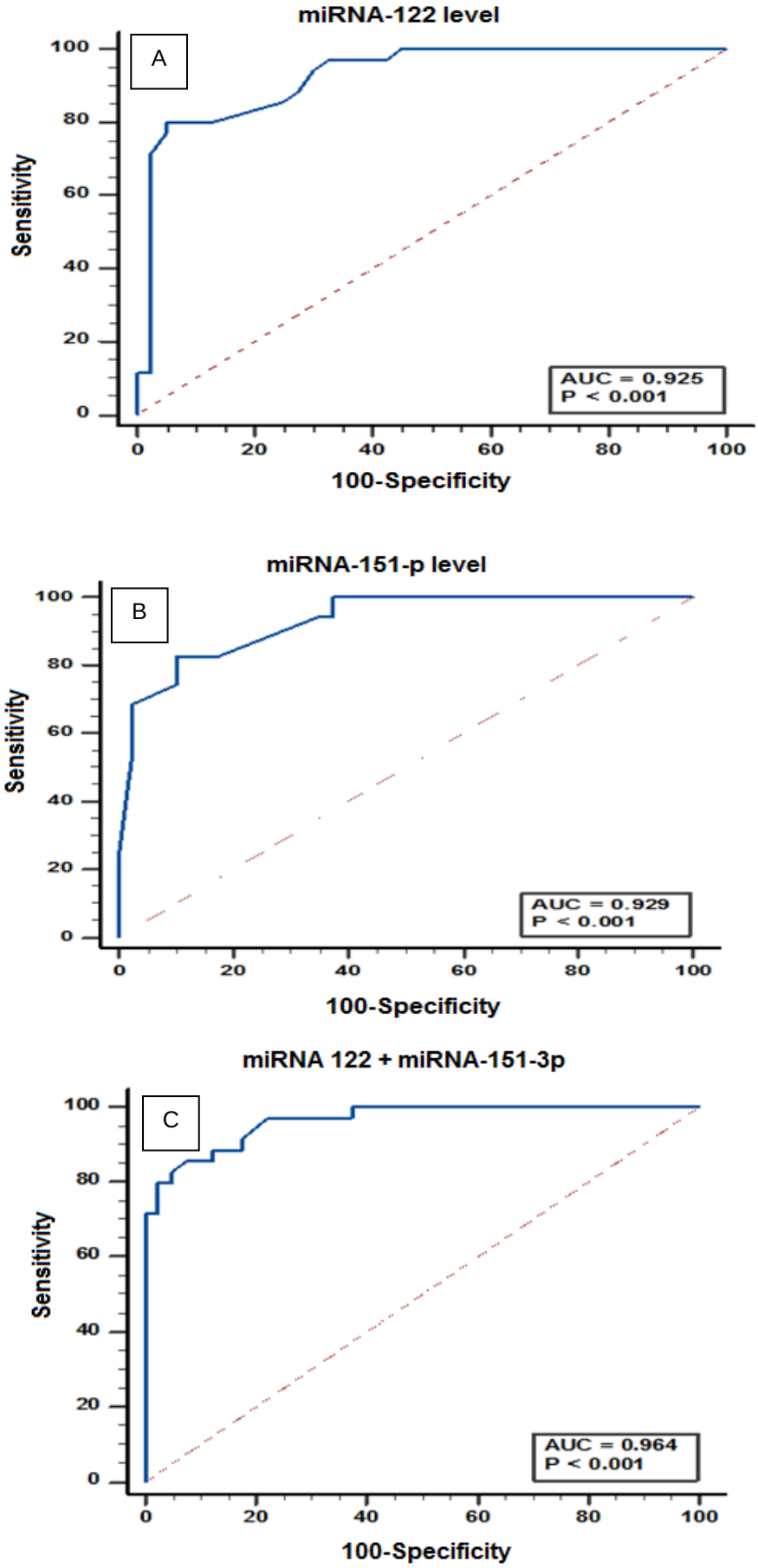


Figure 4: ROC curve of miRNA-122 in (A), miRNA 151-3p in (B), and combined miRNA 122+miRNA 151-3p in (C) to detect coronary artery stenosis in patients

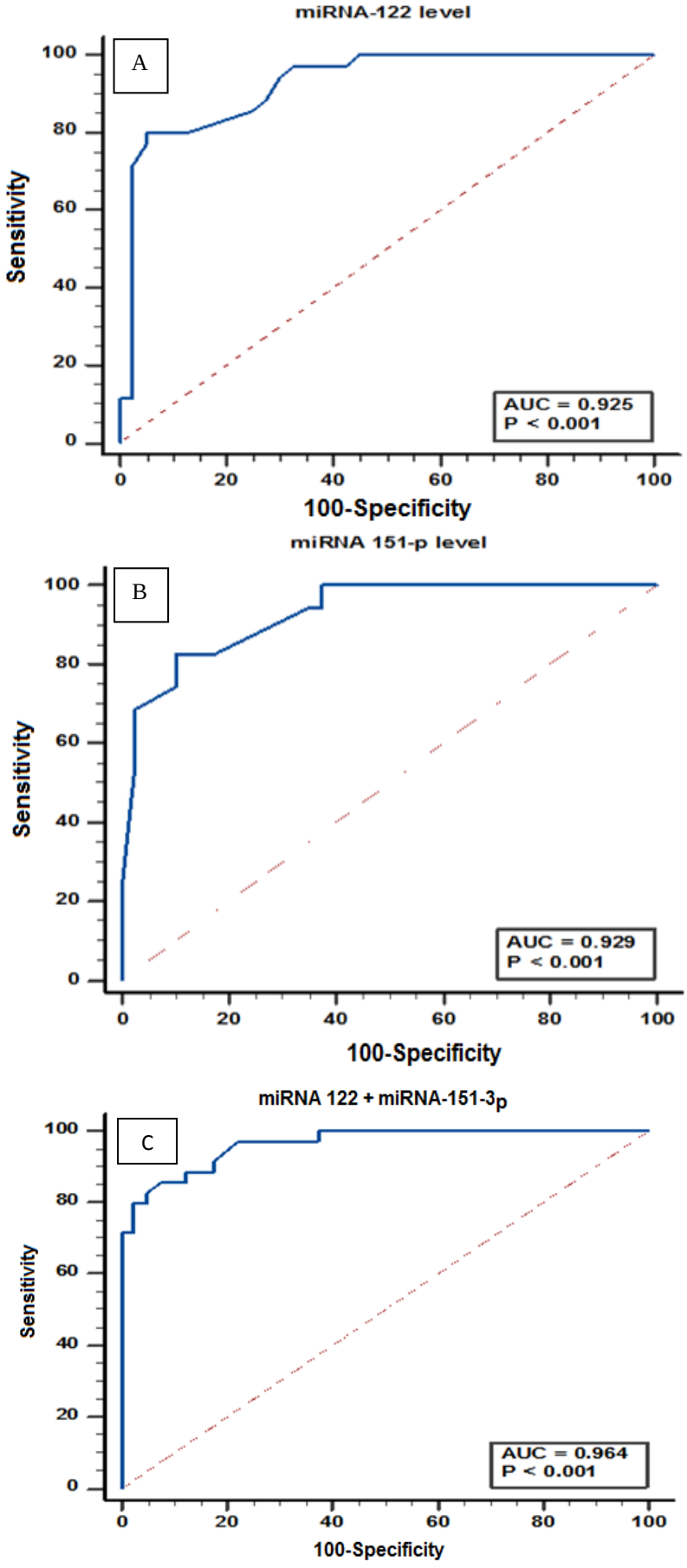


Figure 5: ROC curve of miRNA 122 in (A), miRNA 151-3p in (B), and combined miRNA 122+miRNA 151-3p in (C) to detect control vs. patients group

Table 8: Validity of miRNA-122, miRNA 151-3p, and combined miRNA-122+miRNA 151-3p to detect cut of value control vs. patients group

Parameter	Cutoff value	AUC	Sensitivity	Specificity	P-value
miRNA 122	>1.17	0.996	100%	97.3%	<0.001
miRNA 151-3p	>1.15	0.990	100%	94.7%	<0.001
MiRNA 122+ miRN 151-3p		1	100%	100%	<0.001

Also, miRNA-151-3p showed a good power to distinguish patients with ACE at cut off 1.29 with sensitivity and specificity of 95% and 53.3%, correspondingly, as illustrated in Table 9 and Figure 6. Combination of both miRNA-122 and miRNA-151-3p increases the power with sensitivity and specificity of 92.5% and 68.3%, respectively, as illustrated in Table 9 and Figure 6.

Coronary artery disease (CAD) is a critical public health problem and the main reason for death globally. Although significant advancement occurred in the preceding few decades in the CAD management and its incidence continues to upsurge, signifying the necessity for early diagnosis of CAD [21]. At present, CAD diagnosis depends on electrocardiography (ECG) that was proven to be inadequate. Thus, the efficient revealing of cardiac indicators was planned to overcome the ECG limits. Particularly the cardiac troponin has established to have high sensitivity and specificity in the myocardial damage diagnosis [22] and nonstandard values at any time after chest pain start are highly foretelling of an adverse cardiac event. In our current research, it was found that there was statistically substantial rise of cardiac troponin in NSTEMI and STEMI groups in comparison to controls. Also, noteworthy rise of cardiac troponin in NSTEMI and STEMI groups in comparison to UA group and between STEMI and NSTEMI group. There are some limitations in using troponins to identify acute myocardial infarctions (AMI) as troponins can be raised in other situations as well. For example, tachycardia, shock, and direct blunt trauma to the chest cause injury to the heart. Moreover, viral myocarditis and sarcoidosis increase cardiac troponin levels. [23]. A solo biomarker may be unsatisfactory in diagnosis of CAD or AMI. Therefore, a blend of dissimilar miRNAs could be profitable. Thus, this study aims to focus on the role of circulatory miRNA 122 and 151-3p as diagnostic and prognostic indicators in ACS. In the current study, we have revealed the association of different expression levels of circulating miR-122 and miR-151-3p in ACS patients compared to healthy individuals; correlate their expression levels with coronary artery stenosis. Accordingly, we tried to show the potential of both miRNAs to distinguish patients with ACE from patients without ACE. MiR-122, considered as liver-specific [24], possesses vital roles in lipid metabolism [25], and was related with the incidence and severity of CHD in dyslipidemic patients [26]. Wang *et al.* revealed positive correlation of miR-122 with cholesterol, triglycerides, and atherosclerotic intensity [27]. Numerous key genes tangled in fatty acid biosynthesis and metabolism were proved to be controlled by miR-122, and silencing of miR-122 lead to reduced circulatory levels of total cholesterol and triglycerides [28, 29]. In the current study, the results revealed that circulatory miR-122 levels increased from control group to STEMI group ($p<0.001$), the ROC curve established a good power of miRNA-122 to discriminate patients from the control group at cut off 1.17 with sensitivity and specificity of 100% and 97.3%, correspondingly and showed a similar inclination as cTnI in AMI cases; the circulatory miR-122 positively correlated with cTnI in AMI patients. These findings intensely showed that circulatory miR-122 can be an indicator for ACS diagnosis and the values of elevated miR-122 positively interrelated with the severity of coronary artery stenosis. The ROC analysis established a good power of miRNA-122 to detect coronary stenosis in patients at cut off 1.52 with sensitivity and specificity of 80% and 95%, correspondingly. Our finding was explained as liver-associated miR-122 has been well-known to functionally adjust metabolism of lipoprotein. Consequently, the changed expression of this miRNA is critically tangled in the dyslipidemia

incidence [30]. These results are in accordance with previous research done by Gao *et al.* established that, the increased values of miR-122 was associated with CAD incidence and high miR-122 values positively associate with the severity of CAD [26]. Our data are similar to finding of Yao *et al.* explored that level of miR-122 was overexpressed in patients of AMI contrasted to non-AMI control and cTnI was increased in AMI patients and strongly positively correlated with circulating miR-122-5p [31]. There was significant elevation in mi-RNA 122 level in cases with ACE compared to cases without ACE (p<0.001).

Table 9: Validity of miRNA 122, miRNA 151-3p, and combined miRNA-122 +miRNA 151-3p to detect patients with ACE

Parameter	Cutoff value	AUC	Sensitivity	Specificity	P-value
miRNA 122	>1.4	0.845	85%	75%	<0.001
miRNA 151	>1.29	0.790	95%	53.3%	<0.001
miRNA 122 + miRNA-151-3p		0.851	92.5%	68.3%	<0.001

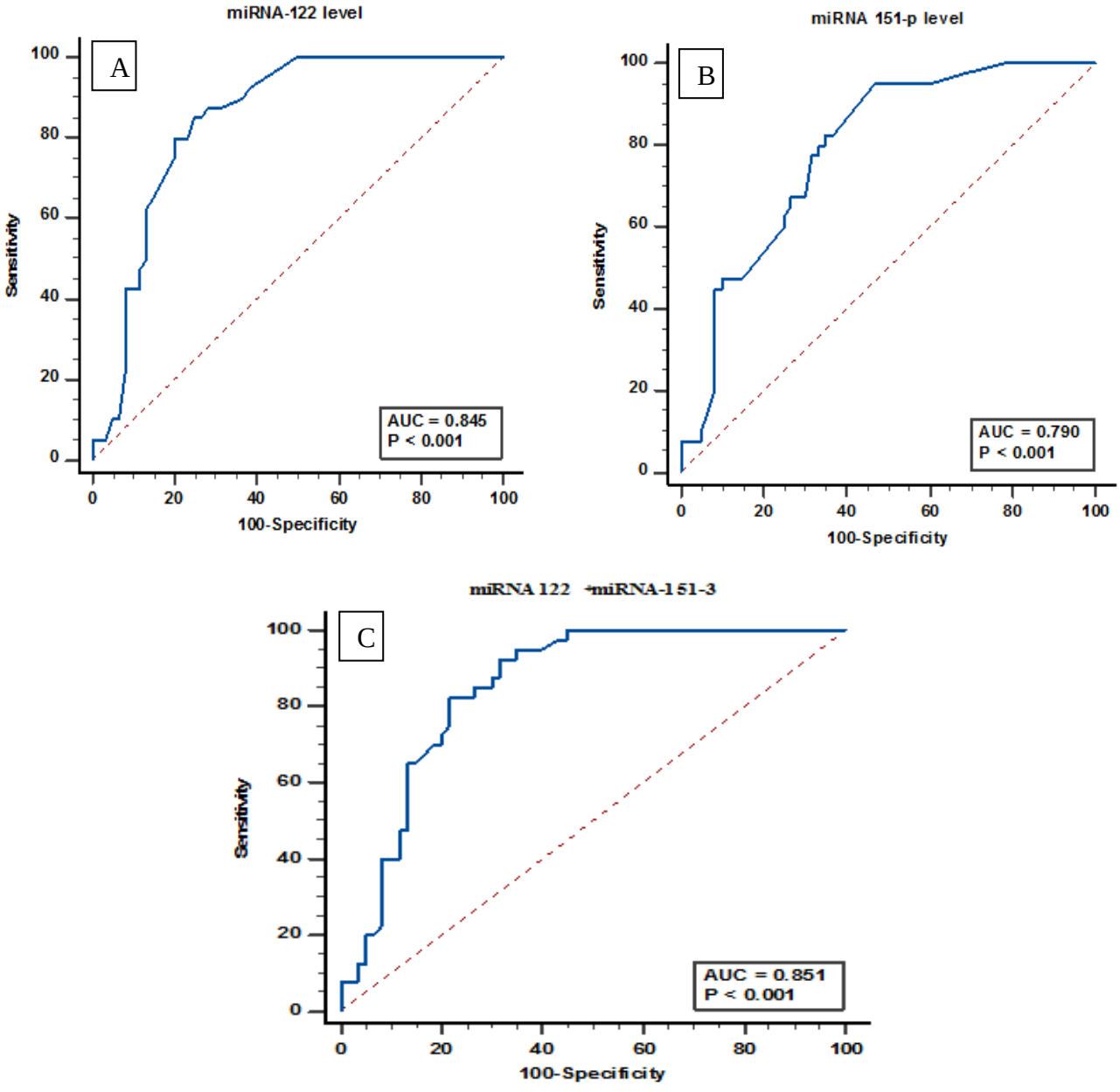


Figure 6: (A) ROC curve of miRNA 122 I and (B) miRNA 151-3p, and (C) combined miRNA 122+ miRNA-151-3p to detect ACE

The ROC curve set a good power of miRNA-122 to discriminate patients with ACE from patients without ACE at cut off 1.4 with sensitivity and specificity of 85% and 75% separately. These data indicate the prognostic role of miR 122 in the ACE prediction in the course of ACS as miR-122 seems to be a direct contributor in the worsening cardiovascular system by stimulating inflammation, oxidative stress, and ECM installation in several cardiovascular diseases [32]. Previous studies obviously enhance the pathophysiological effects of miR-122 in cardiovascular fibrosis and dysfunction. Augmented the expression of MiR-122 aggravates the loss of autophagy and amplified inflammatory reaction, ECM installation, cardiovascular fibrosis, and dysfunction facilitated by Ang II. Above all, the suppression of miR-122 can perform as antifibrotic, anti-apoptotic, anti-inflammatory, and antioxidant functions. Directing the miR-122 has appeared as an early indicator and new therapeutic strategy in contradiction of development of cardiovascular fibrosis and associated diseases and an augmented declaration of cardiovascular roles of the miR-122 will aid to improve current interferences [33]. Considering the finding of Hänninen *et al.* miR 122- 5p was a biomarker for hazard evaluation in cardiogenic shock, it is probable that miR-122-5p might be considered as a general indicator for organ hypoperfusion irrespective of the cause of shock [34]. Badacz *et al.* detected relations between miR 122-5p release and upcoming incidents of CVD and MI. In the current review by Ali Sheikh *et al.*, regarding the therapeutic role of miRs in CAD, miR-122- 5p is proved, to adjust lipoprotein metabolism. Thus, the changed level of miR-122 might be a contributor of dyslipidemia, causing adverse cardiac and neurological ischemic diseases [35]. Actually, in ischemic heart patients, miR-122 level was decreased. The adverse effect of miR 122 is confirmed by the experiment done by Zhang *et al.*, when myocardiac cells subjected to hypoxia display increased miR-122 expression and reduced of microRNA-122 boosts the viability of cardiac cells and opposites the apoptotic outcome of hypoxia via moderating the cell autophagy pathways [36]. Contrary to preceding findings and our results, D'Alessandra *et al.* revealed that circulating levels of miR-122 were lesser in AMI patients compared to healthy individuals [37]. Corsten's research showed that the circulating level of miR-122 was higher in acute cardiac failure, while no noteworthy change was detected in AMI patients [38]. Also, Wang *et al.* established no substantial variance of plasma miR-122 values between AMI patients and control group [39]. The discrepancy of the association between circulating miR-122 value and AMI in dissimilar studies might be partly clarified by the changed criteria of patients included in those studies. The role of circulatory miR-151-3p in plasma samples of ACS subjects is indefinite and narrowly studied. According to our results, there was steadily significant elevation in miRNA-151-3p level from control group to STEMI group ($p<0.001$), there was significant elevation in miRNA-151-3p level in cases with ACE compared to cases without ACE. Also, there was noteworthy positive correlation between mi-RNA 151-3p with cardiac Troponin and coronary stenosis. Also, miRNA-151-3p showed a good power to detect coronary stenosis in patients at cut off 1.56 with sensitivity and specificity of 82.9% and 90%, respectively. Our results are in consistent with Horváth *et al.* who revealed that, miR-151-3p, was considerably up-regulated in STEMI patients [40]. They explained their results by the interaction between miR151-3p and STAT3, which controls the inflammatory reaction in macrophages [41]. This is a reasonable elucidation of its dysregulation in STEMI. Furthermore, the dependency of miR-151-3p level on the amount of endothelial shear stress, a well-identified moderator of atherosclerotic plaque progress and disruption, has been defined [42, 43]. The study of Horvath *et al.* in 2021, is thought to be the only study that study the role of miR 151-3p in ACS and it demonstrate its role in STEMI only. Very little information is available regarding the role of miR-151-3p in ACS, while no association has been identified with coronary stenosis. Several studies have reported the upregulation of miR-151-3p in dissimilar cancer types [17, 18]. The role of miR-151-3p is still debated in atherosclerosis except single study that confirms: Mir 151-3p acting as a protector

against atherosclerosis by inhibiting apoptosis of endothelial cells in in AS by targeting IL-17A [44]. Currently, no cardiac-specific biomarker meets all the standards for an "ideal" biomarker of ACS. No known test is together very sensitive and very specific for AMI, therefore we cannot make the ACS diagnosis depending on a sole cardiac biomarker level found within a few hours following symptom start. ROC curve analysis of our results revealed a good power of combination of both miRNA-122 and miRNA-151-3p with sensitivity and specificity of 100% and 100%, correspondingly, to distinguish patients from the control group. Also, the combination of both miRNA-122 and miRNA-151-3p was done with sensitivity and specificity of 85.7% and 92.5%, respectively, to detect coronary stenosis in patients. Moreover, combination of both miRNA-122 and miRNA-151-3p increases the power with sensitivity and specificity of 92.5% and 68.3%, respectively, to distinguish patients with ACE from patients without ACE. In addition, the combination of miR-122 and miR-151-3p has good effective diagnostic and predictive value than any lone miR. Impending follow-up studies are required to verify their clinical significance.

The limitations of the present study are the small sample size and costly investigation. MiRNA level measurement is not feasible in remote areas due to the unavailability of PCR. Accordingly, recurrent assessment of miRNAs in patients having acute ischemic events can add more evidence on the miRNA action and stability that ought to be assumed in upcoming studies. Future studies in different health centers would be more helpful and can provide a more robust insight into the predictive ability of the miR-122 and miR-151-3p in ACS.

Conclusion

We revealed that the plasma levels of miR-122 and miR-151-3p were considerably increased in ACS patients and were positively correlated with troponin and coronary stenosis. In addition, both miR-122 and miR-151 -3p were diagnostic biomarkers for ACS and were capable of evaluating the degree of coronary artery stenosis and have prognostic value for adverse cardiac events.

Abbreviations

ACS	Acute coronary syndrome
ACE	Adverse cardiac events
UA	Unstable angina
ROC	Receiver operating characteristic analyses
ECG	Electrocardiograph
qRT-PCR	Quantitative real time PCR
PCI	Percutaneous intervention
cDNA	Complementary DNA
NSTEMI	Non-ST-segment elevation myocardial infarction

Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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HOW TO CITE THIS ARTICLE
Heba Fawzy*, Mohammed S. Ghareeb, Hala Mosaad, Implication of Plasma miR 122 and miR 151-3p Levels in Diagnosis and Prognosis of Acute Coronary Syndrome Patients. *J. Med. Chem. Sci.*, 2024, 7(1) 59-77.
DOI: https://doi.org/10.26655/JMCHEMSCI.2024.1.7
URL: https://www.jmchemsci.com/article_179551.html