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Detection of Micro RNA-499 In Acute Myocardial Infarction, Significance Of A New Marker.

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ABSTRACT

The introduction of novel biomarkers in the field of acute coronary syndromes (ACS) is urgently needed to improve diagnostic accuracy; identify subgroups of patients who may benefit from a specific therapeutic modality in the acute phase. The aim of this study is to measure miRNA 499 plasma levels in acute myocardial infarction (AMI) in Egyptian population and compare its diagnostic accuracy with other routine diagnostic tests. The study included 100 patients with AMI and 100 healthy control subjects. Assessment of miRNA 499 level was done for every participant using. SYBER Green based real-time PCR. Level of miRNA 499 was significantly higher in AMI group compared to control group with 100% sensitivity &100% specificity. miRNA 499 is one of the novel cardiac biomarkers & had an important role in diagnosis of AMI.

Keywords: Acute coronary syndromes, Myocardial infarction, Micro-RNA.

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INTRODUCTION

Acute myocardial infarction (AMI) is now considered as one of leading cause of morbidity and mortality all over the world (1).Traditional and routine methods, including an electrocardiogram (ECG), coronary angiography, and circulating markers, are important in the diagnosis of AMI (2) (3). Clinical presentations including chest pain and echocardiographic findings are often nonspecific in patients with AMI (4). Circulating biomarkers including troponins and creatine kinase-MB (CK-MB) are considered now as the most reliable biomarkers for AMI diagnosis. However, both cannot absolutely and accurately confirm or exclude the diagnosis of AMI (1), thus, it is necessary to find novel sensitive and specific biomarkers to improve the prognosis of AMI patients (3).

MicroRNAs (miRNAs) are small, stable non-coding RNAs (< 22 nucleotides) that are implicated in inhibition of mRNA translation or induction of its degradation. miRNAs play major role in most normal cellular events as well as the development of certain human diseases (4) (5).

Because of their relative stability in the circulation, miRNAs are considered potential biomarkers in different diseases. Recently, Different studies support the notion of the implication of miRNAs, especially miRNA499, in the pathogenesis and progression of cardiovascular diseases (6).

The aim of this work was to measure mirna 499 level in patients with AMI and compare its diagnostic accuracy with other routine laboratory tests.

SUBJECTS AND METHODS

This case control study included 100 AMI patients who were admitted to the emergency department of KasrElainy hospital between December 2015 and May 2016, within the first 12 hours of onset of ischaemic-type chest pain lasting for longer than 30 minutes. The patients who had newly developed left bundle branch block, who were admitted more than 12 hours after the onset of chest pain, who had Patients with previous MI or percutaneous coronary intervention (PCI), any hematological disease, acute or chronic infection, significant hepatic dysfunction, kidney failure (glomerular filtration rate (GFR) < 15 mL/min/1.73 m2 or on dialysis), or known or treated malignancies, were excluded from the study. The study also included 100 healthy subjects (normal electrocardiograms and no history of cardiovascular diseases) served as control group (7).
Ethical consideration: Approval was obtained from cardiology department council of Cairo University. Written consent was taken from selected patients . The study was approved by Cairo University research ethics committee and National Research Centre ethics committee. All procedures, included individual data were treated with confidentiality following Helsinki Declaration.

METHODS

Blood specimens (5ml) were collected immediately after admission and within 12 after the symptom onset in the AMI group. All blood samples were collected & divided on 2 EDTA-containing tubes. Thus, 1st tube was centrifuged at 4 °C at 3,000 rpm for 10 min to separate plasma. The supernatants were obtained then CKMB mass was measured using Elecsys (Roche Diagnostics) and 2nd tubes were stored in a −80 °C freezer for further RNA analysis.

RNA analysis:

Total RNA was extracted from EDTA anticoagulated blood using High pure total RNA preparation kit (Roche applied science, Germany), followed by cDNA synthesis for each extracted sample by Transcriptor first strand cDNA synthesis kit (Roche Applied science, Germany).

Expression level of microRNA 499 was measured using SYBER Green based real-time PCR, and consequent relative quantification analysis with the aid of GAPDH as a house keeping gene on LightCycler 2.0 instrument (Roche applied Science, Germany). The 20-uL PCR reaction mixture for the mir 499 & the corresponding housekeeping gene for each sample, included 15 uL of master mix with the following components: 9 uL of PCR grade water, 1 uL of forward primer & the housekeeping gene (20 picomol/uL), 4 uL

of ready to use MasterPLUS SYBER Green I (Roche Applied Science, Germany), & 50 ng of cDNA. The thermal profile was as follows; initial denaturation at 95 °C for 10 min, followed by 45 cycles of amplification, starting by denaturation at 95°C for 10 sec, annealing at 66°C for 20 sec & extension at 72°C for 25 sec. Following amplification, an extra cycle of melting curve analysis was done for product characterization by heating the reaction mixture from 65°C to 95°C at a rate of 0.2°C/Sec. LightCycler 2.0 real time PCR systems software automatically calculates the gene expression values by relative quantitative analysis.

Statistical analysis:

The data will be analysed using Microsoft Excel 2010 and statistical package for social science(SPSS version 24.0) for windows (SPSS IBM., Chicago, IL). Results will be expressed as mean ± SD with 95% confidence interval using medians for quantitative variables; a p value < 0.05 will be considered statistically significant. Pearson correlation coefficient (r) will be done to show the correlation between different parameters in this study. The ROC curve was used to detect the cutoff points and the Correlation between the sensitivity and specificity of MiRNA 192, MiRNA 122 and MiRNA 499. Diagnostic parameters of subjects will be compared using the parametric parameters will be compared using the independent samples (t) test.

RESULTS

Demographic and clinical data of cases and controls were shown in table 1.

Table 1: Descriptive data of studied groups.

Descriptive parameters		Control	Patients	P. value
Age		48.0± 7.9	52.74 ± 6.8	0.8
Sex	Female	54 (54%)	27(27%)	0.09
	Male	46 (46%)	73(73%)	
Diabetes		19 (19%)	23 (23%)	0.1
Hypertension		17 (7%)	37 (73%)	0.08
Diabetic &Hypertension		8 (8%)	55 (55%)	0.03*

The MiRNA 499 of control, ranged between (1.02 - 10.11) with Mean ± S.D (4.75 ± 3.0), while in patient group, it ranged between [(10.75 – 39.5) x 103] with Mean ± S.D (9455.1 ± 929.6), with (P-value < 0.001) that means the values of MiRNA 499 increased or upregulated in patients with elevated cardiac enzymes with highly significant difference (figure1).
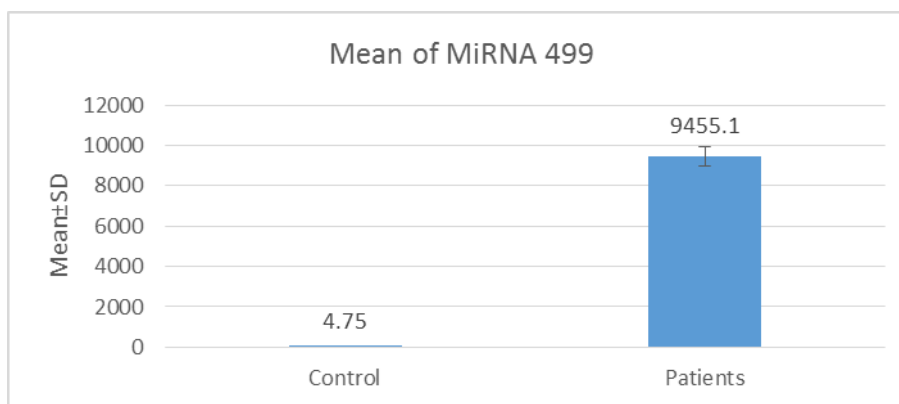

Figure 1.comparison of MiRNA 499 between MI & control groups.

There was a positive correlation (direct proportion) between MiRNA 499with CK MB (r= 0.421; P < 0.001) as shown figure 2.
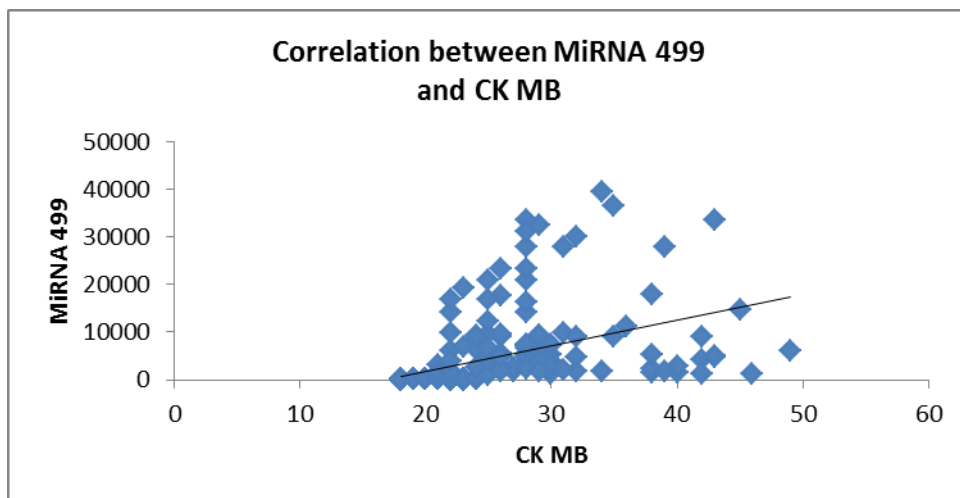

Figure 2. Correlation between MiRNA499 & CK MB

ROC curve for MiRNA 499, at cutoff 10.11 showed 100% sensitivity and 100% specificity. The AUC was 1.0 (P= 0.001, 95% CI). (Figure 3).
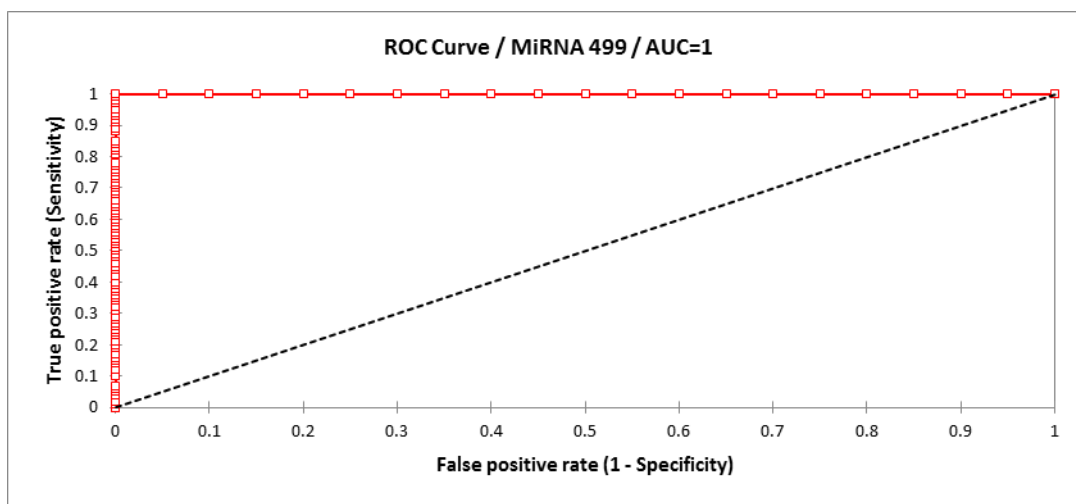

Figure 3 . ROC curve for Mirna 499.

DISCUSSION

Accumulating studies reveal the importance of miRNAs in regulating key signaling and lipid homeostasis pathways that alter the balance of atherosclerotic plaque progression and regression(8) (9) This novel cardiac biomarker will improve diagnostic accuracy of AMI especially in subjects with non- specific elevation of conventional cardiac biomarkers.

This current study has reported significant increase in miRNA499 in blood of AMI patients compared to control group.

The current study describes the role of micro RNA as a new cardiac biomarker that increases significantly in cases of acute myocardial infarction with 100% sensitivity & specificity .This sensitivity &

specificity could be explained by small sample size & the huge difference in micro RNA 499 between the studied groups .

This finding is in agreement with different studies demonstrating role of miRNA499 in AMI patients. (10, 11, 12). Zhang L et al studied 227 patients with chest pain and found that MiR-499 was significantly elevated in 142 patients diagnosed with AMI as compared with 85 patients in non-AMI group and 100 subjects in healthy control group. They found that miR-499 was highly positively correlated with the serum creatine and CK-MB. The area under the curve (AUC) of miR-499 for the diagnosis of AMI was 0.86, with an optimal cut-off value of 4.79, sensitivity of 80%, and specificity of 80.28%.

Shalaby et al measured serum miRNA-499 in a total of 110 patients ; 85 ACS patients (37 UA, 48 NSTEMI) and 25 noncardiac chest pain patients (NCCP). They found that miRNA-499 was significantly increased in UA and NSTEMI patients compared with NCCP patients. ROC curve analysis revealed that the AUC of miR-499 for the diagnosis of UA and NSTEMI was 0.98 and 0.97, respectively.

A meta-analysis of eight studies covering 1634 participants which evaluated the diagnostic value of miR-499 as well as other types of RNA biomarkers of myocardial infarction done by Cheng et al revealed a satisfactory sensitivity and specificity values of MiR-499 (sensitivity: 0.88 (95%CI: 0.86–0.90; $P=0.0000$); specificity: 0.87 (95%CI: 0.84–0.90; $P=0.0000$)) . Among all miRNAs investigated in this study, miR-499 was found to be most significantly associated with myocardial infarction. However, Widera, et al(13), demonstrated that miR-499 is not useful as either diagnostic or prognostic markers in Acute Coronary Syndrome (ACS) & they showed a large overlap between patients with unstable angina or myocardial infarction.

Condorelli G, et al(14), mentioned that increase level of miR-499 (as well as miR-133a/b) is also seen in skeletal muscle injury and therefore are not AMI specific .

As shown above, our results went with the stream of multiple trials that confirm the role of micro RNA-499 as a novel biomarker of myocardial infarction.

We recommend further validation of standardized technique and reference values of micro RNAs level in larger number of patients having not only myocardial infarction but also different cardiac diseases. Measuring the novel cardiac biomarker in different situations that cause non cardiac cause of elevation of conventional cardiac biomarkers is essential to confirm its high specificity.

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