The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90 (2), Page 2839-2846

Serum microRNA-499-5p Expression and Its Correlation with Chemokine (C-C motif) Ligand 18 in Acute Myocardial Infarction

Asmaa R. Abdel-Hamed [a], Elen Nasser [a], Dina M. Abo-Elmatty [a], Gamila M. Nasr [b], Ahmed Salah Salem [b*]

[a] Department of Biochemistry, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt

[b] Department of Cardiology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

***Corresponding author:** Ahmed Salah Salem, **Tel.:** +2 01069966565, **Email:** ahmedsalah@med.suez.edu.eg

ABSTRACT

Background: The major cause of illness and death is acute myocardial infarction (AMI). Therefore, to reduce death rates, effective and precise diagnostic biomarkers are needed.

Objective: The aim of the current study was to evaluate the association between the expression of miRNA-499-5p and the chemokine (C-C motif) ligand 18 (CCL18) in AMI and its related characteristics in Egyptian populations.

Patients and methods: A total of 150 participants were separated into 2 groups for a cross-sectional analytical study; AMI group was composed of 75 patients with AMI, whereas the control subjects' group was made up of 75 people who seemed to be healthy. Over a six-month period from March to September 2018, patients were chosen from the Cardiac Care Units at Suez Canal University Hospital and General Hospital. Using 6-Quantitative Real-Time Polymerase Chain Reaction, the expression of miRNA-499-5p in serum was measured. Using an enzyme-linked immunosorbent test, plasma CCL18 is determined.

Results: Patients with AMI had considerably higher plasma levels of CCL18 than the control group (236.6 vs. 56.05 ng/mL; P<0.001). Significant P-value indicates a positive correlation between plasma CCL18 and male sex, smoking, heart rate, fasting blood sugar, and cardiac markers. In 89.3% of AMI patients, serum miRNA-499-5p expression was significantly increased by 6.36-fold (P<0.001). Creatine kinase MB and cardiac troponin I both exhibited a positive connection with serum miRNA-499-5p expression (P=0.003 and P=0.002, respectively). Additionally, serum miRNA-499-5p expression and plasma CCL18 showed a positive connection (P=0.004).

Conclusion: CCL18 and miRNA 499-5p are potential biomarkers for AMI and possible predictors for the risk of myocardial damage in Egyptians.

Keywords: CCL18, miRNA-499-5p, Acute myocardial infarction, Cross sectional study, Suez Canal University.

INTRODUCTION

Coronary heart disease represents one of the main causes of death rates globally[1]. Atherosclerosis represents one the important risk factors for cardiovascular events. Atherosclerosis is believed to be inflammatory process from onset to progression to plaque rupture[2].

Acute myocardial infarction (AMI), angina pectoris, or ischemia can result from a plaque rupture. Myocyte necrosis that results from AMI triggers a range of biochemical intracellular signaling cascades, including the release of inflammatory cytokines[3].

The extent of myocardial injury, subsequent left ventricular remodeling and clinical prognosis are all determined by inflammatory processes following an acute myocardial infarction and reperfusion[4]. Inflammatory biomarkers have the potential to be good predictors of cardiovascular outcome. Furthermore, they can help in new treatment strategies, such as neutralizing certain leukocyte migration and inflammatory substances, thereby affecting the progression of the illness, and improving heart output after AMI,[4] because atherosclerosis is caused by inflammation and immune cells; it's not surprising that chemokines and chemokine receptors have been related to AMI[5].

Chemokines are small signaling proteins that attract circulating leukocytes to inflamed regions and activate them. Chemokine activate G protein-coupled receptors and stimulate chemotaxis[6,7]. CC chemokines are found at the chronic inflammation sites and tend to attract mononuclear cells[6].

Chemokines has important role in atherosclerosis and subsequent myocardial injury, and remodeling after acute coronary syndrome[8]. In acute cardiovascular diseases, chemokines are involved in inflammation, necrosis, neovascularization, and leukocyte recruitment. At all stages of atherosclerosis, leukocyte recruitment and infiltration are critical. Atherosclerotic lesions express a number of chemokine, such as CCL3/MIP1a, CCL5/RANTES, and CC Chemokine Ligand-18/pulmonary (CCL18/ PARC)[9]. The majority of CCL18/ PARC is released in the lungs, where it attracts T lymphocytes. In reaction to ischemia, this chemokine stimulates fibroblasts, causing lung fibrosis and perhaps cardiac fibrosis[10].

MicroRNAs (miRNAs) have recently attracted increased interest due to the fact that they are commonly deregulated in a variety of clinical disorders, including cardiac illnesses[11]. Endogenous short non-coding RNAs called miRNAs couple with specific places in the 3'

Received: 05/10/2022
Accepted: 08/12/2022

untranslated region of mRNAs for protein-coding genes to control the expression of a variety of genes. miRNAs are 21–25 nucleotides in length. As a result, miRNAs are involved in a variety of physiologic and pathologic functions[12].

miRNAs have a major influence on the expansion of AMI. The new biomarkers miRNA-1, miRNA-133a, miRNA-208, and miRNA-499-5p are used to diagnose AMI[13]. miRNA-499 is encoded by myosin gene family, may prevent the multiplication of cardiomyocyte progenitor cells, which encourages cell differentiation[14]. The aim of the current study was to evaluate the correlation between CCL18 and serum miRNA 499-5p expression in Egyptian patients with AMI. Estimating the circulating levels of these biomarkers would be useful in understanding the disease biology and predicting important prognostic and pathologic parameters in AMI.

PATIENTS AND METHODS

A cross-sectional analytical study included 150 participants, recruited from March 2018 to September 2018. AMI group was composed of 75 patients with AMI, whereas the control subjects' group was made up of 75 people who seemed to be healthy. Patients of AMI group were chosen from the Cardiac Intensive care at Suez Canal University Hospital and Ismailia General Hospital. For each participant, blood pressure and heart rate were assessed.

Inclusion criteria:

Patients diagnosed with AMI, according to the Coordination committee ESC/ACCF/AHA/WHF Task Force on the International Classification of AMI ,[15] and individuals should have good renal function at presentation based on GFR (>60mL/min/1.73m2).

Exclusion criteria:

Patients with recent history less than one year of cardiac catheterization or cardiac surgeries and patients with pericarditis or external cardiac trauma either blunt or penetrating were excluded from this study.

Sample collection and preparation:

On arrival at the Emergency Room, patients had 5ml of peripheral blood obtained (the mean duration from the start of chest discomfort was 96.6 minutes); another sample was taken 8 hours later. Every sample was split into two parts: In order to evaluate CCL18, 3ml of plasma were drawn and placed into potassium EDTA tubes, and 2ml were drawn into plain tubes for serum separation for RNA extraction, analysing fasting blood glucose (FBG), cardiac enzymes [creatine kinase (CK), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), cardiac troponin I (CTnI)] and kidney function (serum creatinine, sodium, and potassium).

Laboratory measurements: CCL18 plasma levels are determined with the Human Pulmonary Activation Regulated Chemokines (PARC/CCL18) ELISA Kit (Shanghai Shanghong (SRB) Biotech Co., Ltd, China, catalogue -No. 201- 12-0067) based on the directions provided by the manufacturer. CCL18 ELISA results were available in 24 hours after sample collection.

The serum was used to measure:

a) FBG utilizing a colorimetric technique (Biodiagnostic, Egypt, Catalog No. GLU109240).
b) Kidney functions including creatinine using a colorimetric technique (Biodiagnostic, Egypt, Catalog No. CR 12 51), and sodium and potassium using ion selective electrode method (Siemens Healthcare Diagnostics Inc. USA, REF S600).
c) Cardiac enzymes including CK, CK-MB, LDH using an enzymatic kinetic method [Siemens Healthcare Diagnostics Inc. USA, (REF DF38), (REF DF32), (REF DF54) respectively], and CTnI using chromatographic- immunoassay method [Abon Biopharm (Hangzhou) Co., Ltd. China, REF CTI-402].

RNA extraction

Through the use of the Qiagen miRNeasy Mini kit, total RNA from serum, including miRNA, has been extracted (Qiagen, Catalog No. 217004). A NanoDrop 1000 spectrophotometer has been used to measure the concentration of RNA (NanoDrop Tech, Wilmington, DE). Using qRT-PCR, the expression of miRNA-499-5p was assessed. A TaqMan® microRNA reverse transcriptase kit performed the reverse transcription (RT) phase (Applied Biosystems, Catalog No.4366596). The following cycling conditions were used for the reactions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. 24 hours after sample collection, miRNA-499-5p PCR results were made available.

Statistics assessment

miRNA-499-5p expression is shown as ΔCT Expression values. By subtracting the CT values of the housekeeping gene (RNU6B) from the target miRNA's CT values, the CT value was calculated (miRNA-499-5p). The relative expression of miRNA-499-5p was evaluated using the conventional $2^{-\Delta\Delta CT}$ approach[16].

Ethical Considerations:

After thoroughly explaining the purpose and methods of the study, all participants were required to provide written consent. The SCU Faculty of Pharmacy in Ismailia, Egypt, received Ethics Committee approval for the current study (Code # 201803MH1). This study was conducted in conformity with the principles of the Helsinki Declaration.

Anytime and without giving a reason, research participants have the freedom to leave the experiment. All participants' privacy and the secrecy of the data acquired were guaranteed.

Statistical Analysis

The collected data were introduced and statistically analyzed by utili/zing the Statistical Package for Social Sciences (SPSS) version 25 for windows. The mean and standard error (SE) of quantitative data were used to depict them, whereas the percentage of qualitative variables was used. Since the data of miRNA-499-5p relative expression did not follow a Gaussian distribution, the median and ranges from the 25th to the 75th percentile were used to calculate miRNA-499-5p levels. Non-parametric methods, such as Mann-Whitney U test for two independent groups and Kruskal-Walli's test for more than 2 categories, were used to measure the expression of miRNA-499-5p for various groups. Using Spearman rank association, correlation analysis was performed (rs). P value ≤0.05 was considered to be statistically significant.

RESULTS

Clinical traits and biochemical assessments of the studied groups: Propensity score matching for case and control group was done and it was found that the two groups are matched regarding all coronary artery disease risk factors (age, gender, smoking, diabetes, hypertension, renal functions, and hemodynamics) (**Table 1**). Moreover, plasma CCL18 was significantly increased in AMI patients in comparison with the control group at P-value <0.05.

Table 1: Demographic data, clinical traits and biochemical assessments of the studied groups.

Variables	Control (n=75)	AMI patients (n=75)	*P*-value
Age (years)	57.73 ± 1.287	56.25 ± 1.238	0.390
Sex			
Male	35 (46.66)	62 (82.66)	
Female	40 (53.33)	13 (17.33)	
Lifestyle			
Smoker	17 (22.66)	59 (78.66)	
Non-smoker	58 (77.33)	16 (21.33)	
FBG (mg/dL)	109.21 ± 1.13	111.83 ± 3.56	0.1
Kidney functions			
Sodium Level (mmol/L)	140.07 ± 0.434	139.28 ± 0.486	0.31
Potassium Level (mmol/L)	4.119 ± 0.072	4.183 ± 0.062	0.23
creatinine Level (mg/dL)	1.09 ± 0.032	1.110 ± 0.059	0.21
Cardiac markers			
CK (IU/L)	144.17 ± 3.08	455.13 ± 27.03	< 0.001
CK-MB (IU/L)	18.15 ± 0.496	202.98 ± 14.6	< 0.001
LDH (IU/L)	165.17 ± 4.452	513.29 ± 20.17	< 0.001
CTnI (ng/mL)	0.196 ± 0.013	1.297 ± 0.05	< 0.001
Heart rate (bpm)	76 ± 1.815	83 ± 1.864	0.298
Blood pressure (mmHg)			
Systolic blood pressure(mmHg)	139 ± 3.6	141 ± 3.8	0.1
Diastolic blood pressure(mmHg)	84 ± 1.113	85 ± 1.505	0.2
CCL18 (ng/mL)	56.05 ± 1.97	236.6 ± 8.8	< 0.001

Data are presented as mean ± SE for continuous variables and as percentages for categorical variables. Comparisons were performed with t-test. AMI; acute myocardial infarction, CK; creatine kinase, CK-MB; creatine kinas MB, CTnI; cardiac troponin I, CCL18; chemokine (C-C motif) ligand18, FBG; fasting blood glucose, LDH; lactate dehydrogenase. P-value <0.05 is significant.

Serum microRNA-499-5p expression and chemokine CCL 18 in acute myocardial infarction:

The Patients' median miRNA-499-5p expression levels were increased by (6.36-fold) compared to controls (P<0.001), with more than their expression at 8 hours. It was significantly higher after presentation than during presentation [34.68 (range 30.19-38.95) vs. 28 (range 26.22-29.9), P-value <0.05]. Study group shows higher CCL18 at presentation than control [0.53 (SD 0.64) vs. 0.50 (SD 0.7), P-value <0.05] more over its expression at 8 hours post presentation was higher than at presentation [0.73 (SD 0.72) vs. 0.53 (SD 0.64), P-value <0.05]. MicroRNA-499-5p level at 31.6 shows 77% sensitivity and 91% specificity for diagnosing AMI as shown in ROC curve (**Figure 1**).

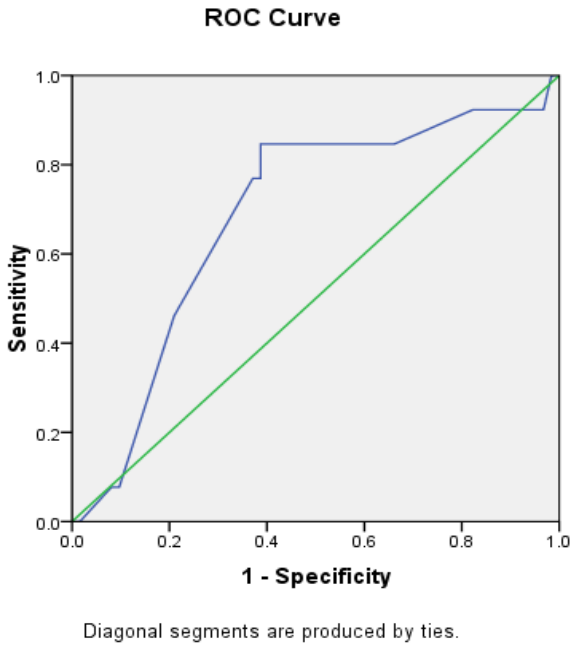

Figure 1: Receiving operator characteristic curve with microRNA-499-5p level at 31.6 shows 77%sensitivity and 91 % specificity for diagnosing acute myocardial infarction (area under the curve= 0.686, P=0.036).

CCL18 level at 0.29 shows 100%sensitivity and 65 % specificity for diagnosing AMI, as shown in ROC curve (**Figure 2**).

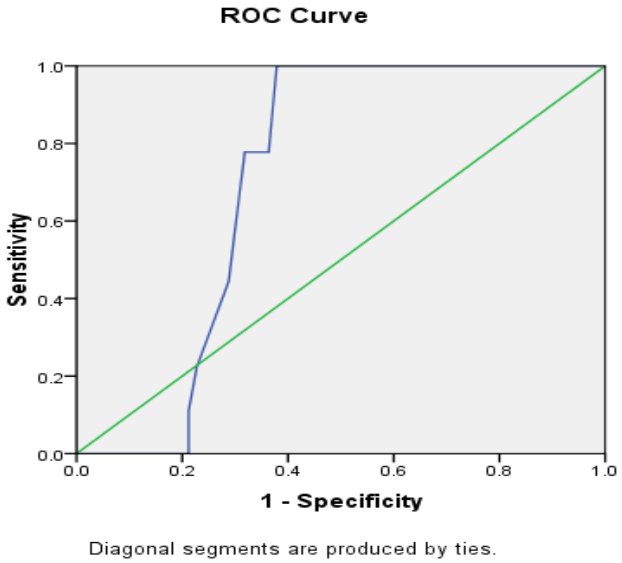

Figure 2: Receiving operator characteristic curve with CCL18 level at 0.29 shows 100%sensitivity and 65 % specificity for diagnosing acute myocardial infarction (area under the curve= 0.711, P=0.041).

Regarding levels of CCL18 and microRNA-499-5p and their correlation with time from onset of symptoms, we found that at 8 hours from presentation CCL18 has non-significant correlation with time from onset of symptoms (R=0.2, P-value 0.1) (**Figure 3**), however microRNA-499-5p has significant correlation with time from onset of symptoms. (R=0.7, P-value <0.001) (**Figure 4**).

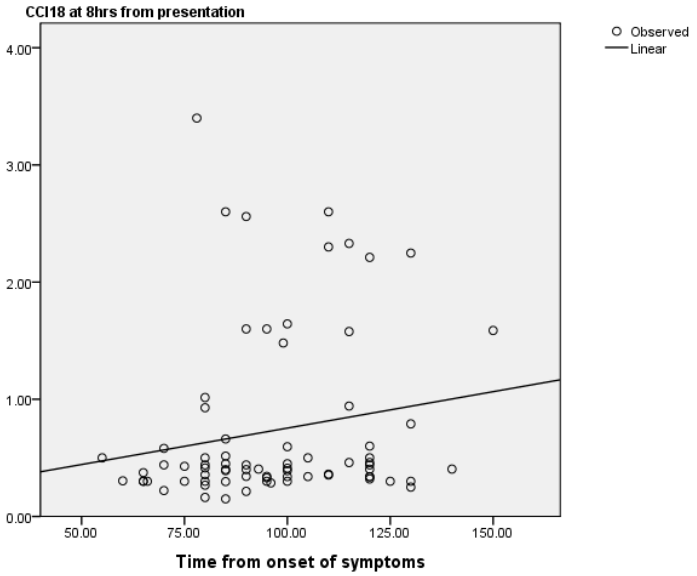

Figure 3: Scatter plot shows correlation between CCL18 at 8 hours from presentation and time from onset of symptoms. (R=0.2, P=0.1).

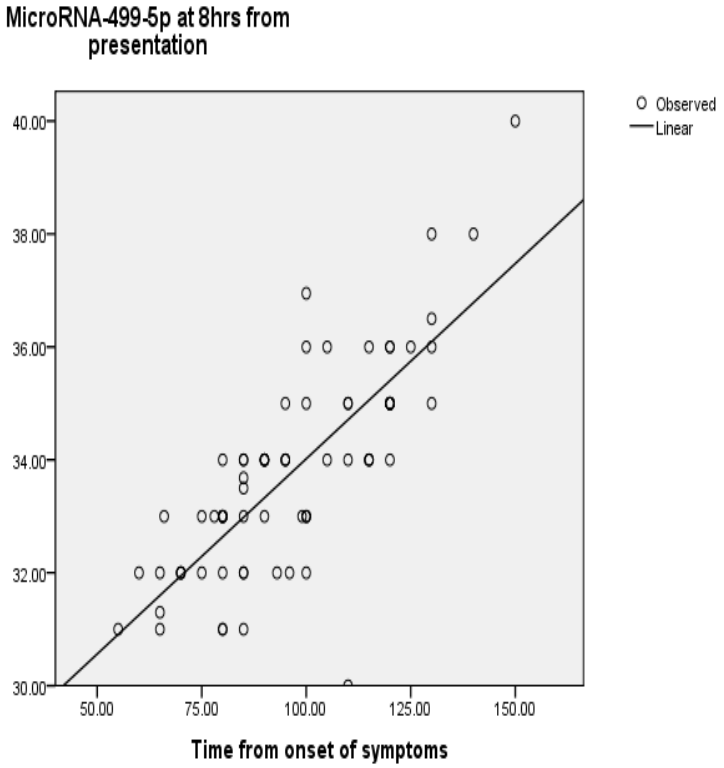

Figure 4: Scatter plot shows correlation between microRNA-499-5p at 8 hours from presentation and time from onset of symptoms (R=0.7, P <0.001).

The analysis of logistic regression to test whether the biomarkers were marked predictors for AMI revealed that CCL18 is a good predictor with odds ratio (OR): 1.4 (95% confidence interval 0.8-2.4, P=0.04), more over microRNA-499-5p shows also a good prediction for AMI with OR: 1.9 (95% confidence interval 1.83-1.99, P=0.032).

Associations between plasma CCL18 and various clinical and biochemical measures in the study group: Plasma CCL18 showed a positive correlation with male sex, smoking, heart rate and FBG level. Additionally, there was a strong significant positive correlation between plasma CCL18 level and all cardiac markers including CK, CK-MB, LDH and CTnI at *P<0.001*. No correlation was observed between CCL18 plasma level and age, kidney functions and diastolic blood pressure (**Table 2**).

Table 2: The levels of plasma CCL18 (ng/mL) and several clinical indicators of the population under study were correlated using Pearson's equation.

Variables	Pearson's correlation coefficient (r)	*P*-value
Demographic data		
Age (years)	0.125	0.127
Sex (male/female)	0.358	<0.001
Smoker/non-smoker	0.488	<0.001
Fasting blood glucose (mg/dL)	0.414	<0.001
Kidney functions		
sodium Level (mmol/L)	0.026	0.752
potassium Level (mmol/L)	0.032	0.695
creatinine Level (mg/dL)	0.080	0.330
Cardiac markers		
CK (IU/L)	0.559	<0.001
CK-MB (IU/L)	0.616	<0.001
LDH (IU/L)	0.730	<0.001
CTnI (ng/mL)	0.717	<0.001
Heart rate (bpm)	0.481	<0.001
Blood pressure (mmHg)		
Systolic blood pressure	0.043	0.006
Diastolic blood pressure	0.035	0.670

AMI; acute myocardial infarction, CCL18; chemokine (C-C motif) ligand18, CK; creatine kinase, CK-MB; creatine kinas MB, CTnI; cardiac troponin I, FBG; fasting blood glucose, LDH; lactate dehydrogenase. P-value <0.05 is significant.

Expression profile MicroRNA 499-5p in patients with AMI: There was no statistically significant difference between patients and controls for the housekeeping gene RNU6B expression across all samples (P=0.21) (**Table 3**). However, there was a difference in the mean ΔCT values of miRNA-499-5P between control and patient groups (P<0.001). In 89% (67/75) of the patient samples, miRNA-499-5p was found to be up-regulated. When compared to the control, the mean average miRNA-499-5p overexpression in patients was noticeably higher (6.36-fold) (P<0.001) (**Table 3**).

Table 3: MicroRNA-499-5p expression levels in acute myocardial infarction patients and control groups

Variables	Control (n=75)	AMI patients (n=75)	*P*-value
Mean CT			
RNU6B	31.3 (30.16-32.58)	32 (29.46-34.97)	0.21
miRNA-499-5p	30 (28-33)	28 (26.22-29.9)	< 0.001
Δ CT	-0.57 (-3.2-1.5)	-3.72 (-5.45- -2.06)	< 0.001
ΔΔ CT		-2.67 (-4.4- -1.01)	
Fold change		6.36 (2.01-21.1)	

Data is presented as the median (1st -3rd quartiles). Mann-Whitney U test was used. CT: threshold cycle number. ΔΔCT and fold change against control were calculated. P-value <0.05 is considered significant.

Correlation between the expression level of miRNA-499-5p and the demographics, clinical traits, and biochemical markers of patients with AMI.

Age, blood pressure, or heart rate had no effect on the expression of microRNA-499-5p, although men and smokers had greater expression (**Table 4**).

Table 4: Association between miRNA-499-5p expression and clinical characteristics of acute myocardial infarction patients

Variables		No.75	Fold change	*P*-value
Age	≤ 60 years old	54 (72)	6.1016 (1.647-21.62)	0.981
	> 60 years old	21 (28)	6.382 (2.033-20.31)	
Sex	Male	62 (82)	10.84 (2.25-65.46)	0.0243
	Female	13 (18)	4.436 (1.95-19.93)	
Lifestyle	Smoker	58 (77)	10.89 (2.25-50.41)	0.025
	Non-smoker	17 (23)	4.436 (1.95-18.38)	
Heart rate	≤ 70 bpm	21 (28)	3.289 (1.44-15.05)	0.096
	> 70bpm	54 (72)	8.2775 (2.866-22.61)	
Blood pressure (mmHg)	Normal (120-129)/ (80-84)	16 (21)	7.940 (2.61-22.6)	0.583
	High (130-139)/ (85-89)	17 (23)	8.731 (2.61-21.6)	
	Type I (140-159)/ (90-99)	20 (27)	3.014 (1.117-27.3)	
	Type II (160-179)/ (100-109)	17 (22)	2.73 (1.73-7.68)	
	Type III ≥180)/ (≥110)	5 (7)	8.74 (0.809-37.5)	

Data is presented as the median (1st -3rd quartiles). Mann-Whitney test was used for age, sex, life style and heart rate and Kruskal-Wallis for blood pressure. P-value <0.05 is significant.

Levels of CCL18, CK-MB, and CTnI were significantly positively correlated with serum miRNA-499-5p expression. However, there was no obvious correlation among FBG, CK, LDH, or renal function (**Table 5**).

Table 5: Association between acute myocardial infarction patients' miRNA-499-5p expression and various biochemical tests

Variables	Spearman's rho correlation coefficient	*P*-value
CCL18 (ng/mL)	0.294	0.004
FBG (mg/dL)	0.13	0.999
Kidney functions		
Serum sodium (mmol/L)	-0.076	0.516
Serum potassium (mmol/L)	0.018	0.879
Serum creatinine (mg/dL)	0.018	0.879
Cardiac markers		
CK (IU/L)	0.002	0.987
CK-MB (IU/L)	0.385	0.003
LDH (IU/L)	0.21	0.07
CTnI (ng/mL)	0.421	0.002

CCL18; chemokine (C-C motif) ligand18, CK; creatine kinase, CK-MB; creatine kinas MB, CTnI; cardiac troponin I, FBG; fasting blood glucose, LDH; lactate dehydrogenase. P-value <0.05 is significant.

DISCUSSION

AMI represents an important cause of death worldwide, diagnostic biomarkers are required to decrease incidence of death. Troponin is one of the most common AMI biomarkers. Circulating troponin levels increase three hours after the beginning of chest discomfort as a result of the delayed release period of troponin. Biomarkers with greater sensitivity and specificity than troponin are needed[17]. The study's objective was to determine the circulating levels of the chemokine CCL18 and miRNA-499-5p as non-invasive biomarkers for AMI early diagnosis. Additionally, the study aimed to correlate blood levels of CCL18 and miRNA-499-5p to AMI structural features. MicroRNAs have recently been correlated to the pathophysiology of cardiac diseases[18].

The plasma levels of miRNA-1, miRNA-133a, miRNA-133b, and miRNA-499-5p are elevated in AMI patients, suggesting that they could be utilized as biomarkers for heart disease[19-21]. This study showed a 6.36-fold increase in expression of serum miRNA-499-5p

compared to control people, which were correlated significantly with AMI. Additionally, the results showed a significant positive association between the serum blood levels of the established cardiac biomarkers CTnI and CK-MB and serum miRNA-499-5p expression.

In agreement with our study, ***Yao et al.*** and ***Cheng et al.*** discovered that miRNA-499-5p was expressed at higher levels in myocardial infarction patients compared to those with other traditional AMI biomarkers, and that these levels were related positively with circulating CK-MB and CTnI[22,23]. As a result, miRNA-499-5p can be considered an early and specific biomarker that is nearly recognized in the blood 1 hour after the beginning of heartburn[24].

In angiogenesis and hematopoiesis, chemokines act as mediators[25-27]. A circulating chemokine called CCL18/PARC promotes in inflammation, the recovery from damage, and the normal homing of mononuclear blood cells[28-30]. In atherosclerotic plaques, CCL18/PARC is expressed, especially in regions with decreased stability[29,31,32].

According to this study, AMI patients' plasma CCL18 levels were higher significantly than healthy group. A high association between CCL18 plasma level and all cardiac biomarkers was also demonstrated by the study. Myocyte necrosis, which triggers a cascade of biochemical intracellular signalling events including inflammatory cytokines, is the cause of the rise in CCL18 in AMI. Smoking, which is regarded as a key contributor to cardiovascular disease, and plasma CCL18 revealed a significant positive connection in our research. Similarly, ***Sajedi Khanian et al.*** found that males who smoke have greater plasma CCL18 levels[33].

Our research discovered a statistically positive association between the plasma level CCL18 and serum miRNA-499-5p expression in AMI patients. When cardiac myocytes are damaged, miRNA-499-5p, which is expressed in myocytes, can be released into the bloodstream[34]. CCL18 is also released in circulation due to myocytes necrosis which stimulates the release of inflammatory cytokines. Therefore, both miRNA-499-5p and CCL18 may be used as early diagnostic biomarkers of AMI.

LIMITATIONS

Our study was retrospective design, single center, small sample size, also only two samples for each patient 8 hrs. apart however if there were more serial samples taken will give higher efficacy to evaluate our proposed biomarkers, also there was some time lag between samples collection and results, further studies are needed with larger sample size and more frequent serial samples to validate the conclusions from this study.

CONCLUSIONS

AMI patients had significantly higher serum expression of miRNA-499-5p and plasma levels of CCL18, which were positively linked with serum CTnI and CK-MB. Plasma CCL18 level and miRNA-499-5p expression were highly related in AMI patients. Therefore, this study suggests that both miRNA-499-5p and CCL18 are potential early diagnostic AMI biomarkers.

List of Abbreviations:

1. Acute myocardial infarction (AMI)
2. chemokine (C-C motif) ligand 18 (CCL18)
3. Ischemic induced myocardial injury (IMI)
4. Micro RNAs (miRNAs)
5. Enzyme linked immunosorbent assay (ELISA)
6. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)
7. Reverse transcription (RT)
8. Complementary DNA (cDNA)

- **Funding:** No fund from any financing organization.
- **Declaration of conflicting interests:** no compromised interests.
- **Data availability statement:** The article that was published contains the data that were created and examined throughout this investigation.
- **Authors' contributions:** Data collection, analysis, interpretation, and paper writing were all done by all authors.

REFERENCES

1. **Thygesen K, Alpert J, White H (2007):** Universal definition of myocardial infarction. Journal of the American College of Cardiology, 50(22):2173-95.
2. **Libby P, Tabas I, Fredman G *et al.* (2014):** Inflammation and its resolution as determinants of acute coronary syndromes. Circ Res.,114(12):1867-79.
3. **Sutton M, Sharpe N (2000):** Left ventricular remodeling after myocardial infarction pathophysiology and therapy. Circulation,101(25):2981-8.
4. **Penn M (2008):** The role of leukocyte-generated oxidants in left ventricular remodeling. Am J Cardiol., 101(10A):30D-33D.
5. **Braunersreuther V, Pellieux C, Pelli G *et al.* (2010):** Chemokine CCL5/RANTES inhibition reduces myocardial reperfusion injury in atherosclerotic mice. J Mol Cell Cardiol., 48(4):789-98.
6. **Charo I, Taubman M (2004):** Chemokines in the pathogenesis of vascular disease. Circulation Research, 95:858-66.
7. **Rothenbacher D, Müller-Scholze S, Herder C *et al.* (2006):** Differential expression of chemokines, risk of stable coronary heart disease, and correlation with established cardiovascular risk markers. Arterioscler Thromb Vasc Biol., 26(1):194-9.

8. **Parissis J, Adamopoulos S, Venetsanou K *et al.* (2002):** Serum profiles of CC chemokines in acute myocardial infarction: possible implication in postinfarction left ventricular remodeling. J Interferon Cytokine Res., 22(2):223-9.
9. **Hagg D, Olson F, Kjelldahl J *et al.* (2009):** Expression of chemokine (C-C motif) ligand 18 in human macrophages and atherosclerotic plaques. Atherosclerosis, 204(2):e15-20.
10. **Atamas S, Luzina I, Choi J *et al.* (2003):** Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts. Am J Respir Cell Mol Biol., 29(6):743-9.
11. **Sayed D, Abdellatif M (2011):** MicroRNAs in development and disease. Physiol Rev., 91(3):827-87.
12. **Shrivastava S, Mukherjee A, Ray R (2013):** Hepatitis C virus infection, microRNA and liver disease progression. World J Hepatol., 5(9):479-86.
13. **Adachi T, Nakanishi M, Otsuka Y *et al.* (2010):** Nishimura K, Hirokawa G, Goto Y et al. Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin Chem., 56(7):1183-5.
14. **Chen C, Yang S, Chaugai S *et al.* (2014):** Wang Y, Wang DW. Meta-analysis of Has mir- 499 polymorphism (rs3746444) for cancer risk evidence from 31 case-control studies. BMC Med Genet., 126:15.
15. **Kristian Thygesen, Joseph S, Allan S *et al.* (2012):** Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction. Third universal definition of myocardial infarction. Circulation,126(16):2020-35.
16. **Livak K, Schmittgen T (2001):** Analysis of relative gene expression data using real time quantitative PCR and the 2-ΔΔCt method. Methods, 25(4):402-8.
17. **Xin Y, Yang C, Han Z (2016):** Circulating miR-499 as a potential biomarker for acute myocardial Infarction. Ann Transl Med., 4(7):135-9.
18. **Schulte C, Zeller T (2015):** microRNA-based diagnostics and therapy in cardiovascular disease-Summing up the facts. Cardiovasc Diagn Ther., 5(1):17-36.
19. **Kuwabara Y, Ono K, Horie T *et al.* (2011):** Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circulation: Cardiovascular Genetics, 4:446-54.
20. **Condorelli G, Latronico M, Cavarretta E (2014):** microRNAs in cardiovascular diseases: current knowledge and the road ahead. J Am Coll Cardiol., 63(21):2177-87.
21. **Olivieri F, Antonicelli R, Spazzafumo L *et al.* (2014):** Santini G, Rippo MR, Galeazzi R et al. Admission levels of circulating miR-499-5p and risk of death in elderly patients after acute non-ST elevation myocardial infarction. Int J Cardiol., 172(2):e276-8.
22. **Cheng Y, Tan N, Yang J *et al.* (2010):** A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond), 119(2):87-95.
23. **Yao Y, Du J, Cao X *et al.* (2014):** Plasma levels of microRNA-499 provide an early indication of perioperative myocardial infarction in coronary artery bypass graft patients. PLOS ONE, 9(8):e104618.
24. **Zhang L, Chen X, Su T *et al.* (2015):** Circulating miR-499 are novel and sensitive biomarker of acute myocardial infarction. J Thorac Dis., 7(3):303-8.
25. **Mantovani A (1999):** The Chemokine system: redundancy for robust outputs. Immunol Today, 20(6):254-7.
26. **Coelho A, Hogaboam C, Kunkel S (2005):** Chemokines provide the sustained inflammatory bridge between innate and acquired immunity. Cytokine Growth Factor Rev., 16(6):553-60.
27. **Strieter R, Burdick M, Gomperts B (2005):** CXC chemokines in angiogenesis. Cytokine Growth Factor Rev., 16(6):593-609.
28. **Hieshima K, Imai T, Baba M *et al.* (1997):** A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 alpha/LD78 alpha and chemotactic for T lymphocytes, but not for monocytes. J Immunol., 159(3):1140-9.
29. **Schutyser E, Richmond A, Van Damme J (2005):** Involvement of CC chemokine ligand 18 (CCL18) in normal and pathological processes. J Leukoc Biol., 78 (1):14-26.
30. **Lambert M, Sachais B, Kowalska M (2007):** Chemokines and thrombogenicity. Thromb Haemost., 97(5):722-9.
31. **De Sutter J, Struyf S, Van de Veire N *et al.* (2010):** Cardiovascular determinants and prognostic significance of CC Chemokine Ligand-18 (CCL18/PARC) in patients with stable coronary artery disease. J Mol Cell Cardiol., 49(5):894-6.
32. **Sin D, Miller B, Duvoix A *et al.* (2011):** Serum PARC/CCL-18 concentrations and health outcomes in chronic obstructive pulmonary disease. Am J Respir Crit Care Med., 183(9):1187-92.
33. **Sajedi Khanian M, Abdi Ardekani A, Khosropanah S *et al.* (2016):** Correlation of Early and Late Ejection Fractions with CCL5 and CCL18 Levels in Acute Anterior Myocardial Infarction. Iran J Immunol., 13(2):100-13.
34. **Zhao C, Cheng G, He R *et al.* (2015):** Analysis and clinical significance of microRNA-499 expression levels in serum of patients with acute myocardial infarction. Genet Mol Res., 14(2):4027-34.