Expression of microRNA-133a and microRNA-208b in acute myocardial infarction; A randomized clinical trial

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Keywords: CVD, Acute Myocardial Infarction, STEMI, miR-133a and miR-208b, cTnI, CK-MB

Posted Date: June 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2995851/v1

Abstract

Background: Myocardial microRNAs (Myo-miR) like MiR-1, miR-133 and miR-208 are specific to cardiac muscles development and function. Diagnostic potential of MiR-1, miR-133 and miR-208 in acute episodes of myocardial infarction is unknown.

Methods: Patient of newly onset of acute myocardial infarction with elevated ST-segment admitted to Ain-Shams university hospital Cairo from May 2013 to December 2022 were enrolled. Written consent was obtained. Circulating MiRNAs were measured at 04, 08, 12, 24, 48 hours from onset of angina by RT-PCR and compare with conventional creatin-kinases for diagnostic potential. Results were analyzed by student t-test, ROC curve calculations and ANOVA were performed using GraphPad-Prism-9Version.

Results: 746 patients admitted with STEMI and 10(1.34%) cases were presented with new-onset episodes of STEMI with mean±SD age 54.2±8.49 year. miR-133a peaks at 8 hours, miR-208b peaks at 12 hours from onset of AMI compared to cTnI and CK-MB peak at 12hrs($P<0.001$). ROC-curve for miR-133a AUC was 0.583,0.8,1,0.78 and 0.58 at 4,8,12,24 & 48 hours respectively. AUC for miR-208b was 0.87,0.888,0.888,0.627 compared to AUC of cTnI concentrations 0.59,1,1,0.75,0 and (ROC) curve for CK-MB was 0.59,1,1,0.8&0.73 respectively. Positive correlation was present between miR-133a and cTnI ($R=0.926$-$R^2= 0.858$ $P=0.47$), miR-208b and cTnI ($R=0.8$-$R^2=0.64$ $P=0.1$) , miR-208b and CK-MB was ($R=0.888$-$R^2=0.789$ $P=0.044$) and CK-MB-cTnI ($R=0.72$) respectively.

Conclusion: We showed miR-133a and miR-208b diagnostic specificity superior over conventional blood biomarkers for acute onset of STEMI. (NCT05692752)

Introduction

Cardiovascular diseases (CVDs) is one of the leading causes of morbidity and mortality worldwide, including Egypt. Recent reports indicate that Egypt is one of the Eastern Mediterranean region with high burden of CVD (1).

Acute coronary syndrome (ACS) results from inadequate supply of blood flow from the coronary arteries to the heart. Myocardial Infarction (MI) could be divided into two groups, ST segment elevation myocardial infarction (STEMI), a serious form of heart attack in which the coronary artery is completely blocked, resulting in cardiac injury and necrosis; and non-ST segment elevation myocardial infarction (NSTEMI) represents less myocardial tissue damage. .Circulating biomarkers are essential for the diagnosis, prognosis, monitoring and treatment of MI patients, along with electrocardiogram, echocardiography and coronary angiography (2).

Cardiac enzymes are useful for diagnosis and prognosis of MI patient and act as the traditional, convential circulating biomarkers, such as. Ccardiac troponin, (cTn), are currently considered the biomarker of choice for the diagnosis of myocardial infarction (MI) and necrosis because it is the most sensitive and specific available biochemical marker of myocardial ischemia/necrosis.. M Studies,

demonstrated that plasma cTn content is elevated in cardiovascular diseases (CVDs) other than AMI, with or without necrosis (3). Troponins are proteins that regulate muscle contraction. Isoforms of two of these proteins are T and I, which are specific to the myocardium. Cardiac troponin is expressed in both skeletal and cardiac myocytes. Increased serum cTnI level is the gold standard for diagnosing AMI. Troponins levels are most useful to detect the true positive diagnostic sensitivity starting 12 h or more after onset of acute MI episode (4). In the early hours following MI, myoglobin is more sensitive (3).

Myoglobin is a biomarker for early detection cardiac injury because the serum level of myoglobin rises in the first 30 min after the onset of an acute event. Creatine Kinase (CK) is also an enzyme that catalyzes the reversible transformation of Creatine and ATP to creatine phosphate and ADP (4).

In humans, less than 2% of genes can be transcribed into messenger RNA that encodes proteins, while non-coding RNA can be divided into short non-coding RNA (< 200 bp, including microRNA (miRNA) and circular RNA) and long non-coding RNA (lncRNA, > 200 bp). Currently, there are more than 2000 human miRNAs that have been found, which seem to regulate about 60% of human genes (5). MiRNAs regulate cardiac development, remodeling and regeneration, endothelial function, vasculogenesis and neoangiogenesis through a variety of pathways (6). Almost 1000 of miRNAs are regulating 30% of genes within the human genome. MiRNAs are endogenous, noncoding single stranded RNA molecules of 19–22 nucleotides in length, that regulate gene expression at the posttranscriptional level frequently by targeting 3’-UTR of mRNAs with consequent translational inhibition in physiological functions, development and disease, such as cancer, autoimmune and inflammatory diseases, neurodegenerative and CVDs (5).

Extracellular miRNAs in blood are packed in vesicles. They are protected from endogenous ribonucleases to keep their stability. The circulating miRNAs in blood are detected as disease biomarkers. They are used as mediators of cell-cell communication (6).

MiRNAs act as small players in gene regulation helping only to fine tune gene expression. MiRNAs are one of the Epigenetic factors, including DNA methylation, regulate almost all cellular events including cell proliferation, differentiation, and development.

MiRNAs that bind mRNA at 3’- UTR, prevent translation or promote degradation of the mRNA, thus negatively regulating gene expression at the post-transcriptional level (7). miRNAs are responsible for certain cellular signaling / communicating proteins formation. It is believed that acute onset of MI is associated with certain kinds of miRNAs which could be novel diagnostic markers for MI.

Three miR-133 genes have been identified in the human genome, miR-133a-1, miR-133a-2 and miR-133b which are located on chromosomes 18,20 and 6 respectively.

MiR-133 inhibits the progression of cardiac hypertrophy and cardiac fibrosis, promoting the reprogram of cardiac fibroblasts. MiR133a overexpression leads to protect cardiac progenitor cells from apoptosis targeting pro apoptotic genes and inhibiting hypertrophy. Interestingly, it alleviates arrhythmia by regulating the expression of some ion channels. In the same time, it also aggravates electrical activity

disorders, causing atrial fibrillation (AF) and sudden death (8). Starting to evaluate the Diagnostic and Prognostic Value of plasma miRNA133a. in stable coronary artery disease (CAD) and, particularly, in the acute coronary events, where miR-133 levels decrease in the myocardium and increase in the circulation proportionally to the extent of the infarcted area. The value of circulating levels of miR-133 as a diagnostic and prognostic biomarker in these patients during the acute ischemic episode and after primary revascularization has already been established (9).

MiR-133 and miR-1 play a significant role in arrhythmia, MI, diabetic cardiomyopathy, cardiac hypertrophy, cardiomyocyte differentiation and cell reprogramming .MiR-133a- is specifically expressed in heart and skeletal muscles. MiR-133 plays major roles in the developing heart on cell differentiation into muscle tissue and also in later stages of cardiac morphogenesis .The levels of miRNAs (miR-1, miR-133a, amiR-499 and miR208) in the ischemic myocardium after MI were up-regulated (10).

F Wang et al., 2013, demonstrated that miR-133a was released into blood from injured myocardium in the early phase of AMI (12). As mMany preclinical studies results showed that some of miRNAs such as miR-1, miR-133a/b and miR-208 are involved in the heart development, regulate heart and dysregulated in the hypertrophic and failing hearts and some CVDs, including MI. MiR-133 was recently shown its role involved in cardiovascular regenerative medicine directing tissue repair and cellular differentiation (11, 13).

MiR-208 is one of the most important cardiac-enriched miRNAs that play a crucial role in cardiovascular health and diseases. MiR-208 has 2 subfamilies, miR-208a and miR-208b, are encoded within an intron of α-cardiac muscle myosin heavy chain gene (α-MHC, MYH6) which required for cardiomyocyte hypertrophy and fibrosis. And expression of intron of β-cardiac myosin heavy chain gene (β-MHC, MYH7) in response of stress and hypothyrodism. Both these are located on human chromosome 14 (11).

Many preclinical studies showed that some of miRNAs such as miR-1, miR-133a/b and miR-208 are involved in the heart development, regulate heart and dysregulated in the hypertrophic and heart failure events and some CVDs, including MI. MiR-133 were recently showed its role in cardiovascular regenerative medicine inducing tissue repair and cellular differentiation .

MiR-1, miR-133 and miR-208 have been found to be cardiac muscle-specific, and thus have been called myo-miRs (12).

In this study, we investigated circulating level of miR-133a and 208b at different time intervals (4, 8, 12, 24, and 48h) from the onset of the infarction and compare it with the conventional AMI biomarkers cTnI and CK-MB,

Method

Patients:

Patient admitted at Ain-Shams university hospital Cairo from May 2013 to December 2022 of both gender with age 35–60 years attending cardiovascular emergency department of university hospital with acute myocardial infarction (MI) **Table.1**. Patients with previous history of MI were not included. New onset of myocardial infarction with ST-segment elevation (STEMI) on electrocardiogram (ECG) recorded were included in STEMI group. Patient diagnosed with STEMI with history of previous treatment of MI, chronic diseases, renal diseases were excluded from study. Healthy participants were recruited for this study with corresponding to age group of the study. Informed consent was obtained before admission.

Blood samples were collected following the onset of chest pain at 04, 08, 12, 24 and 48 hours and serum was collected from blood samples for analysis of circulating mRNAs and cardiac enzymes. Blood samples were processed within 1 h of collection by two-step centrifugation. The supernatant was transferred to RNase/DNase-free cryotubes and stored at- 80˚c. Plasma is preferred over serum for miRNA analysis to minimize procedural variation caused by differences in clotting and subsequent collection of serum (15). As for CK-MB and cTnI analyses, serum was used as indicated by the manufacturer‘s protocol. Plasma samples were used for miR-133a and 208b quantification. Samples were collected in EDTA vacutainers, since plasma samples containing heparin as an anticoagulant can interfere with downstream assays, such as RT-PCR (16).

Table.1: Characteristics of STEMI Patients

Characteristics	Percentage n(10)
Male	07 (70%)
Female	03(30%)
Age	
35–55	06(60%)
> 55Y	04(40%)
Hypertension	06(60%)
Diabetes	04(40%)
Dyslipidemia	02(20%)

Total RNA isolation

Qiagen miRNeasy Serum/Plasma Kit purification of total RNA was used for analyzing miRNA from plasma samples. miRNeasy Serum/Plasma Spike-In Control for normalization and was injected within the steps of RNA isolation to be used as an internal control. Then prepare reverse transcription reaction and Quantification by PCR Amplification.

CK-MB and cTnI analysis

DRG® Troponin I Elisa (cTnI) (EIA-2952) and DRG® Creatine Kinase (MB-Isoform) (EIA-4112) kits were used for the quantitative determination of cTnI and CK-MB in human serum samples, respectively.

Data Analysis:

Data collected was categorized into two groups miRNA and conventional blood biomarkers. Values were recorded as Mean ± SEM. Both groups were analyzed by t-test employed to assess the association with STEMI of all readings. Separate tests were performed for each category. Receiver Operating Characteristics (ROC) curve and Area Under Curve (AUC) was determined to evaluate associated relation with in two groups. ANOVA was employed to see the statistical significance of variables. Analysis of data was performed using GraphPad Prism 9 software (GraphPad Software Inc., CA, USA).

Results

Plasma levels of circulating miR-133a & miR-208b over 48 hours in AMI patients

Using median-normalized data, circulating miR-133a and miR-208b, showed similar time-point dependent concentration profiles. MiR-133a levels peaked after 8 hours then started declining to reach low near control levels after 48 hours, being 2.65 folds higher relative to the controls; miR-208b peaked after 12 hours and showed high levels in plasma that persisted after 48 hours being 26 folds higher in patients relative to the control (Fig. 1).

Figure 1: Plasma levels of miR-133a and miR-208b in AMI patients showing the peak levels of miR-133a after 8 hours and the peak of miR-208b at 12 hours after the onset. Significance here indicates comparison with control.

To assess the diagnostic potential of both miRNAs, receiver operating characteristic curves (ROC) were drawn for the fold change values of all patients at different time points and the area under the curve (AUC) was calculated for each graph. For miR-133a and miR-208b, the AUC curves were drawn after different hours (Fig. 2& Fig. 3).

Figure 3: Receiver operating characteristic (ROC) curve for miR-208b fold change values discriminates between various time points after the onset of AMI and control group set arbitrarily as 1. AUC is calculated 0.87,0.888,0.888,0.627 and 0.642 at 4,8,12,24&48 hours respectively.

Serum cTroponin I Levels over 48 hours in AMI patients

cTnI levels measured after 4, 8,12,24,48 hours of MI were 2.4, 12, 15, 8.8 and 4.1 folds, respectively, compared with that of control respectively (mean ± SEM, 8.46 ± 2.35) (Fig. 4). cTnI concentrations in AMI patients and controls were also plotted and compared by unpaired samples (t-test). ROC curves were

drawn for the concentration values of all patients at different time points to assess the diagnostic ability of cTnI at each time interval and the area under the curve (AUC) was calculated for each graph. The AUC for values after 8 and 12 hours were both 1 indicating excellent diagnostic ability of cTnI for AMI after 8 and 12 hours from the onset of symptoms (Fig. 4).

cTnI fold change calculated as concentration of patients normalized to mean concentration of controls at different time points, error bars represent SEM. ANOVA p = 0.0025

Serum CK-MB levels over 48 hours in AMI patients

CK-MB concentrations also exhibited a gradual increase at 4, 8, 12, 24 and 48 hours with a 21.68, 39.72, 46.11, 14.26&5.058 mean fold increase respectively creatinine kinase MB fold change at different time points calculated as concentration of serum CKMB normalized to the mean concentration of controls and shown in the figure as mean fold change, Mean ± SEM (25.36 ± 7.69). Error bars represent SEM. P < 0.0001 (Fig. 5a). One Way ANOVA test, the CK-MB concentrations at 4, 8, 12, 24 h were statistically significant relative to the controls (Fig. 5b). ROC curves were drawn for the concentration values of all patients at different time points to assess the diagnostic ability of CK-MB at each time interval and the area under the curve (AUC) was calculated for each graph. The AUC for values after 8 and 12 hours were both 1 indicating reliable diagnostic ability of CK-MB for AMI after 8 and 12 hours from the onset of AMI (Fig. 6) .

Comparing the levels of circulating miR-133a, 208b, cTnI and CK-MB in AMI patients

In 10 patients and 10 controls, miR133a, miR-208b, cTnI and CK-MB were measured in the plasma samples and the corresponding serum sample respectively. The three had similar pattern in blood. The plasma levels of miR133a reached the expression peak after 8hr from the onset of infarction which is 4 hours ahead of the cTnI and CK-MB peak time as they both reached their peak after 12 hours from the MI onset, the same time of miR-208b peak (Fig. 8).

Figure 8: Expression pattern of circulating miR-133a ,208b, cTnI and CK-MB in AMI patients at different time points. Data were normalized to the peak level that miR-133a, 208b, cTnI and CK-MB achieved in each patient ,error bars represent the SEM. On average miRNA 133a achieved a 33.11 ± 15.59 peak fold change 8 hours after the AMI onset. MiRNA 208b peaks reached at 12 hours achived 50.42 ± 17.60. cTnI and CK-MB, both achieved a peak of 15 ± 2.9 after 12 hours 46.11 ± 7.602 12 hours after the same.

The correlation analysis

Figure 9. Correlation between miR-133a&cTnI,R = 0.926 R^2 = 0.858

P = 0.0235. Positive correlation between miR-133a and cTnI.

Figure 10. Correlation between miR-133a&CK-MB, R = 0.424 R^2 0.18 P = 0.47

No significance correlation between miR-133a and CK-MB

Figure 11. Correlation between miR-208b&cTnI, R = 0.8 R^2 = 0.64 p = 0.1

Positive correlation between miR-208b and cTnI.

Figure 12. Correlation between miR-208b&CK-MB, R = 0.888 R^2 = 0.789 p = 0.044

Positive correlation between miR-208b and CK-MB.

Figure 13. Correlation between CK-MB&cTnI, R = 0.732

Positive correlation between CK-MB and cTnI.

Discussion

It is estimated that the human genome encodes more than mature 2000 miRNAs (miRBase v. 22) according to GENCODE data (v. 29), including isoforms with slight variations. Circulating miRNAs are more stable and resistance to degradation by endogenous RNase activity than intracellular miRNAs, reside in microvesicles including exosomes, microparticles and apoptotic bodies, which may provide protection from RNase activity. The stability of circulating miRNAs has stimulated interest in their use as biomarkers for the diagnosis and prognosis of various diseases including CVD (13).

Nevertheless, the property of cTn and CKMB for early diagnosis of AMI is weak because the level of these biomarkers elevates late after MI. Thus, a biomarker that could be detected at the early stage of AMI with a better diagnostic value is needed to compensate for the deficiency of cTn and CKMB. B Wang et al., 2021, study aimed to compare the combined miRNAs could compensate for the deficiency of single miRNA in sensitivity or specificity for an optimal clinical value. Also the results displayed that some miRNAs reached peak time earlier and showed a shorter time window than the conventional biomarkers despite the different collection times of initial blood samples. Some of them were shown to be more valuable than classical biomarkers for the early diagnosis of AMI, and these miRNAs appeared to have the most potential biomarkers within 4 h of the onset of symptoms except miR-133a/b and miR-208b (14).

Muscle- and cardiac-specific miRNAs as miR-1, miR-133a/b and miR208a/b have already been described as deregulated in human MI in previous studies. In the present study, we evaluated miR133a and miR208b as potential circulating biomarkers for AMI. Increases in serum miR-133a level were observed in patients with evidence of cardiac injury (STEMI) after 8 h but unlike miR-208b level which remained elevated after 12 h. MiR-133a and miR-208b returned to baseline levels within 48 h after treatment.

Y Wexler et al.,2020, meta-data analysis comparing between different studies to determine the diagnostic value of miR-133a level whether it may serve as a biomarker for very early detection of AMI, and to evaluate the queried role that it may be useful in distinguishing STEMI from NSTEMI, as the commonly

used biomarkers for the diagnosis of AMI, such as cardiac troponins and CK-MB-, were not effective at very early diagnosis of AMI (within 0–3 hours (15). It is approved in our study as well, as mir-133a started as its peak earlier at 8 hours and before miR-208b and clinical biomarkers TnI and CK-MB as well, their peaks at 12 hours.

It is known that miR-1, miR-133a, miR-133b, miR-206, miR-208a, miR-208b, and miR-499 are muscle-specific miRNAs. MiR-1, miR-133, and miR-208, being involved in heart development and disease. Among them, miR-133b and miR-206 are expressed only in skeletal muscle and miR-208a is expressed only in cardiac muscle. In this study, they showed upregulation of miR-208 levels and downregulation of miR-1 and miR-133 levels from autopsy samples of infarcted heart tissue from patients with MI (16).

In a larger cohort comprised of patients with acute coronary syndrome (ACS), the plasma miR-133a and miR-133b levels were independently associated with increased high sensitive cardiac troponin T (hsTnT) levels. MiR-133a levels were significantly associated with the risk of death, also reported fold increase of miR-133 level in plasma from AMI patients (17). A positive correlation was also reported in the elevated miR-133 and cTnI. This report also indicated that miR-133 may be superior to cardiac troponin I due to some confounding factors that may affect troponin I(TnI) levels. It shows up in the end-stage renal disease, the lower glomerular filtration would increase the troponin I level in the plasma (18). Commonly to our results, CK-MB and cTnI, achieved a peak of 15 ± 2.9 after 12 hours, 46.11 ± 7.602 12 hours, consecutively after the same onset.

In agreement with other related published reports (Kuwabara et al., 2011; C. Widera *et al.*, 2011), related to the correlation between miR-133a level, CPK and TnT in CVD patients. Their results examined that the serum miR-133a Level was sensitive, and was significantly higher in ACS patients compared with non-ACS patients. The serum levels of these miR-133a peaked before 3 hours after chest pain was reported and decreased gradually thereafter, with no elevation of CPK and cTnT. MiR-133a expression levels decreased gradually expression levels decreased (18, 19).

In C. Widera *et al.*, 2011, study including patient cohort with NSTEMI or STEMI, presented with higher levels of miR133a, and miR-208b compared with patients with unstable angina (UA) (18).

YU et al., 2018 study, investigated the expression of miR-133a level in patients with or without AMI following radical surgery for gastric cancer, and to explore its underlying mechanisms. The results suggested that patients with AMI exhibited significantly increased expression of endothelial injury markers and miR-133a compared with patients without AMI (20).

The same for the other MiRNAs in this study including 133a.The levels of the MiRNAs are decreased once the patients reached the hospital, might be used as a biomarker for AMI but it did not show any advantages over cTnT for AMI diagnosis. They are established biomarkers for the diagnosis of AMI and reflect infarct size. Compared with cTnI, cTnT is more specific and sensitive for the diagnosis of AMI compared to miRNAs in related studies. They performed ROC curve analyses to determine the diagnostic values of the four circulating miRNAs and to compare them with cTnT. They hypothesized that cTnT

might be released from necrotic myocardium at the time of the AMI in patients prior to the onset of chest pain (3).

Corsten et al., 2010 study results indicated that the plasma levels of miR-208b in AMI patients, was increased more than 1000 times folds compared with healthy subjects. The changes in plasma level of miR-208b were consistent with cTnT, demonstared that miR-208b was released from injured cardiomyocytes like cTnT (21).

O Gidlöf et al., they demonstrated the levels of plasma miR-208b increased thousands fold in case of STEMI patients compared to healthy controls within 12 h after infarction, correlated with peak troponin I (cTnI), indicating a possible role for circulating miR-208b as a biomarker in diagnosis of STEMI at day 1(2).

Han et al., 2015, study, the plasma miRNA-208 level was significantly higher in AMI group within 12 hours of the MI onset than normal control group. Correlation analysis showed that miRNA-208 had significant correlation with cTnT, but not with CK-MB (23). In our study, miR-208b had significant correlation with CK-MB.

C Li et al., 2015 study aim to compare the expression of different miRNAs including miR-208b in AMI patients. MiR-208b was expressed at a high level in AMI compared to controls. MiR-208b was more effective in patients with NSTEMI. Though other studies there were no significance of the levels of plasma of miR-208b between STEMI and NSTEMI, including ours, especially STEMI (24).

X Liu et al.,2017 study involved 3 groups of the following, AMI patients divided to (one, two, three –vessel CAD), unstable angina (UA) patients, and controls were consecutively included in this study. The AMI patients who sustained percutaneous coronary intervention (PCI) were followed up at 6 months post-AMI. The concentration of miR-208b was significant in the AMI patients than in the other two groups, and it was positively correlated with the levels of CK-MB and cTnI, compared to our results. In addition, the miR-208b concentration in AMI patients with three vessel coronary artery disease (CAD) was higher than that of single- or two-vessel CAD AMI patients. Also, the miR-208b expression after PCI was significantly lower than before, except those with left ventricular remodeling/MACEs was higher after PCI (25).

In this pilot study, M Alavi-Moghaddam et al,.2018, they demonstrated the role of miR-208b as a candidate biomarker for AMI diagnosis and the potential of circulating miR-208b as a prognostic biomarker of 6-month survival in AMI patients (26).

K AGIANNITOPOULOS et al., 2018 study group consisted of Greek AMI patients and controls. All of the AMI patients were sustained a PCI. The relative expression of miR-208b and miR-499 were elevated in AMI patients compared to that of controls (27).

YQ Li ·et al., 2013 study, they demonstrated that circulating miR-208b levels was not significant between STEMI and NSTEMI patients. The same for the other MiRNAs in this study including 133a.The levels of the MiRNAs are decreased once the patients reached the hospital, might be used as a biomarker for AMI

but it did not show any advantages over cTnT for AMI diagnosis. They were still trying to conform which more superior early diagnostic biomarker in case of MI case (28).

In other related published clinical studies (Peng *et al.*, 2014; Boštjančič et al.,2018), they found that the expression of miR-133 was distinctly increased in STEMI and NSTEMI cases compared to non-AMI cases. E. Boštjančič et al.,2018, they demonstrated that miRNAs may contribute to the development of arrhythmias. Their studies included patients who had died after 24 h of MI and others after 1–7 days after MI. Some of them are proven ventricular fibrillation (VF) and others without VF. They were compared to healthy trauma victims were included as control. Their results suggested that in patients with MI with VF, were observed down-regulation of miR-133a/b, even stronger 2–7 days after MI. miR-208 was up-regulated in remote myocardium irrespective of the presence of VF (29).

B Wang et al,2021 systematic review, comparing miRNAs and Conventional Biomarkers as CK-MB and cTn, their results are different in several studies, regarding the potential cofounders such as single miRNA or combined miRNAs, sample size, collection time of samples and Detection Methods (30, 31).

Han et al., 2015, study, including AMI patients, the plasma miRNA-208 level was significantly higher in AMI group within 12 hours onset after than normal control group. Correlation analysis showed that miRNA-208 had significant correlation with cTnT, but not with CK-MB (15).

Declarations

Acknowledgments

Limitations No follow up records are available for the recruited patients. Author contributions Concept—SIH,HM, KA and MZG; design—SIH; supervision—KA, SIH and MZG; fundings—Dina Osama and Ahmed Hegazy.; materials— Dina Osama and Ahmed Hegazy; data collection and/or processing— Dina Osama and Ahmed Hegazy; analysis and/or interpretation— Dina Osama and Ahmed Hegazy. SIH and KA. Literature review—SIH,HM ,KA and MZG; writing—SIH; critical review— SIH,HM ,KA and MZG. Compliance with ethical standards Conflict of interest. The authors declare that they have no conflict of interest. Ethical approval German University Cairo ethics committee [Chair of Committee Prof. Dr. Hans-Georg Breitinger, Hans-Georg Breitinger (hans.breitinger@guc.edu.eg)], approved the study protocols. Informed consent Consent by all participants (patients and healthy controls).

Ethical Approval

All subjects were informed of the nature of the study and thus written consent. Data and code availability that abided by the Helsinki declaration was obtained from all of them and Author's contribution statements. The study was approved by the local ethics committee of both the Ain Shams, Faculty of Medicine, and the German University in Cairo. Informed consent Consent by all participants (patients and healthy controls).

Competing interests

we certify that there is no potential *conflict of interest* in relation to this article.

Authors' contributions

(applicable for submissions with multiple authors)

Funding

No funding grants support this study.

Availability of data and materials

Data and code availability that abided by the Helsinki declaration was obtained from all of them and Author's contribution statements.

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Figures

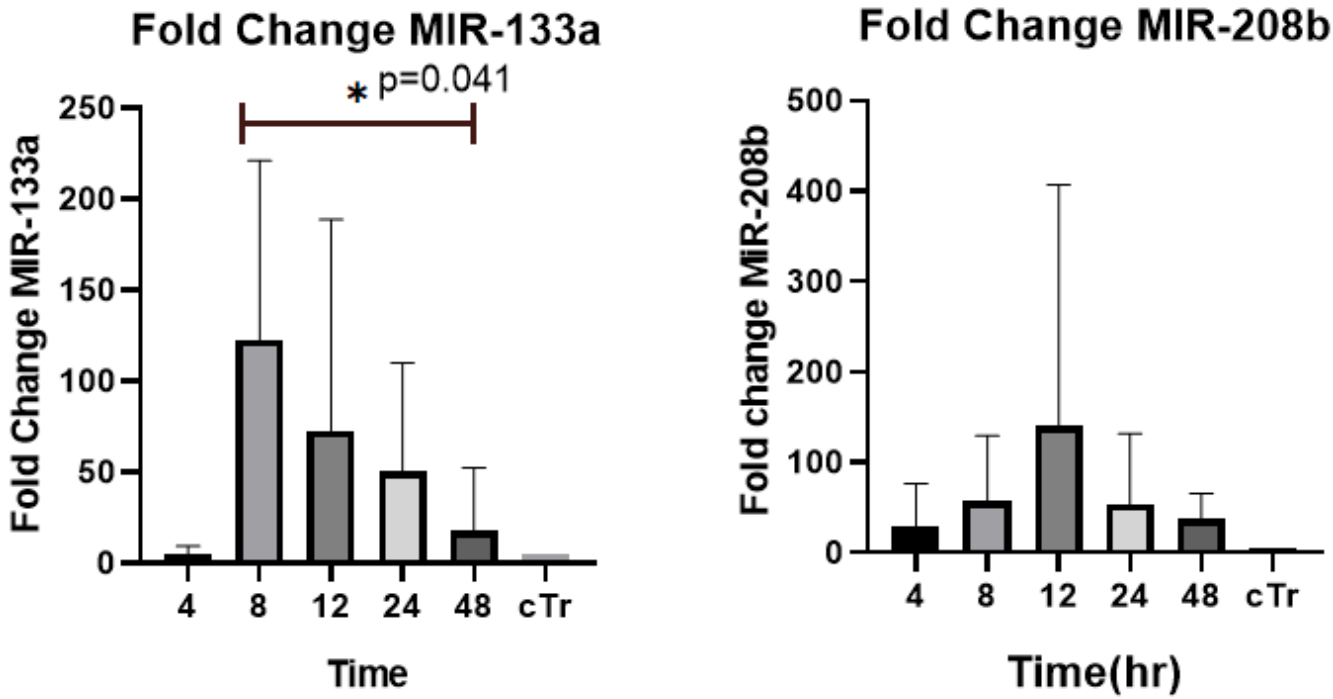

Figure 1

Plasma levels of miR-133a and miR-208b in AMI patients showing the peak levels of miR-133a after 8 hours and the peak of miR-208b at 12 hours after the onset. Significance here indicates comparison with control.

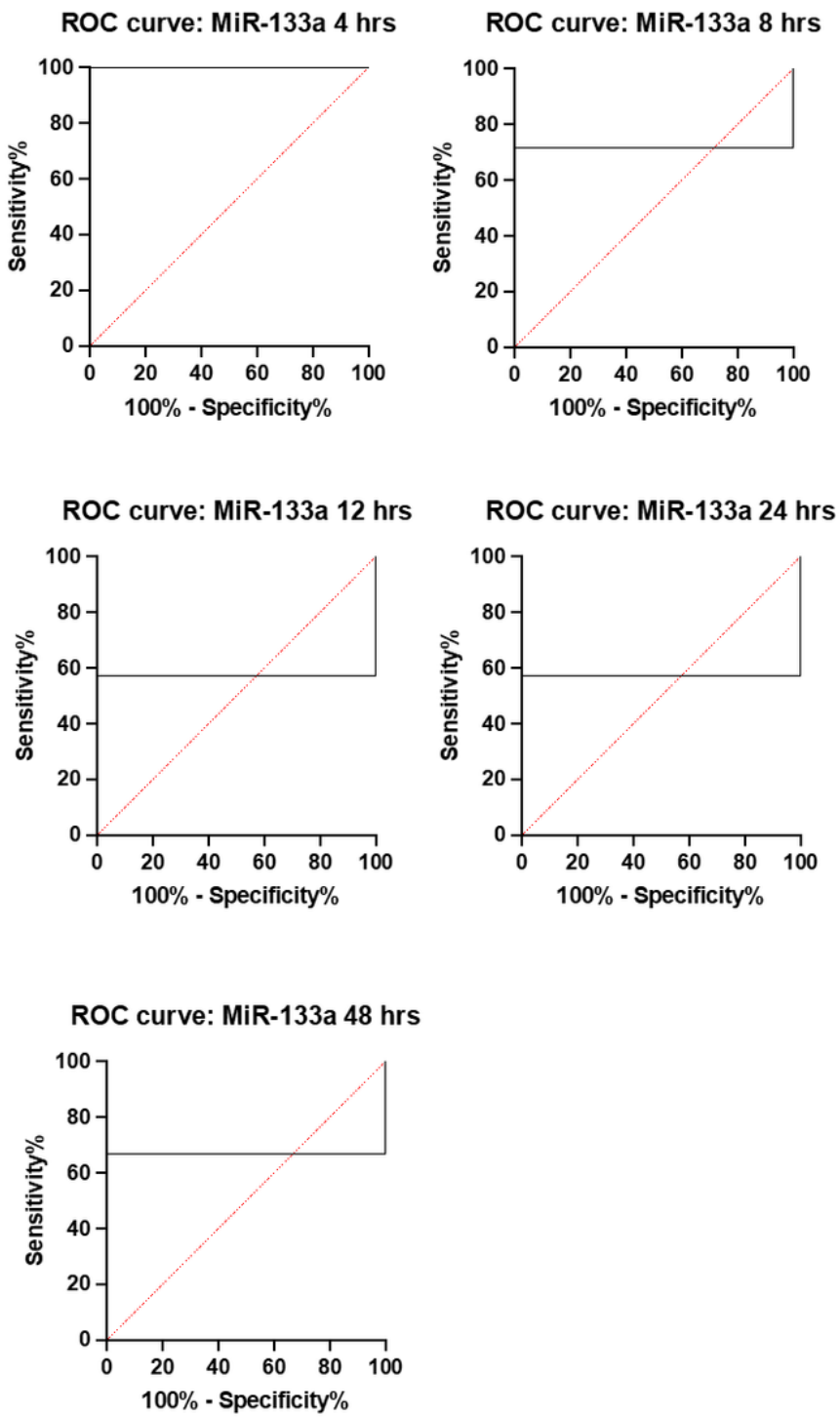

Figure 2

Receiver operating characteristic (ROC) curve for miR-133a fold change values discriminates between various time points after the onset of AMI and control group set arbitrarily as 1. AUC is calculated 0.583,0.8,1,0.78 and 0.58 at 4,8,12,24&48 hours respectively.

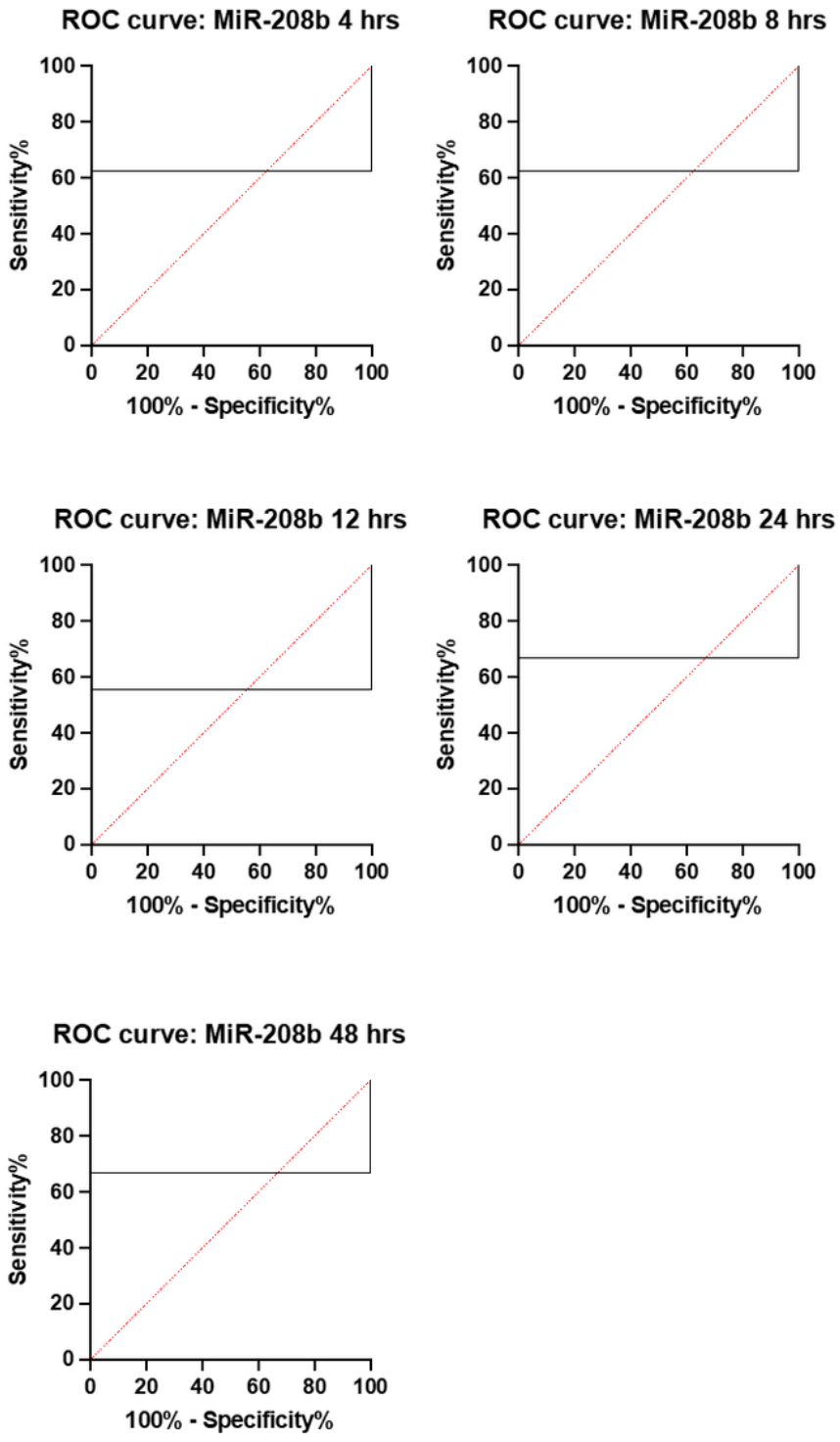

Figure 3

Receiver operating characteristic (ROC) curve for miR-208b fold change values discriminates between various time points after the onset of AMI and control group set arbitrarily as 1. AUC is calculated 0.87,0.888,0.888,0.627 and 0.642 at 4,8,12,24&48 hours respectively.

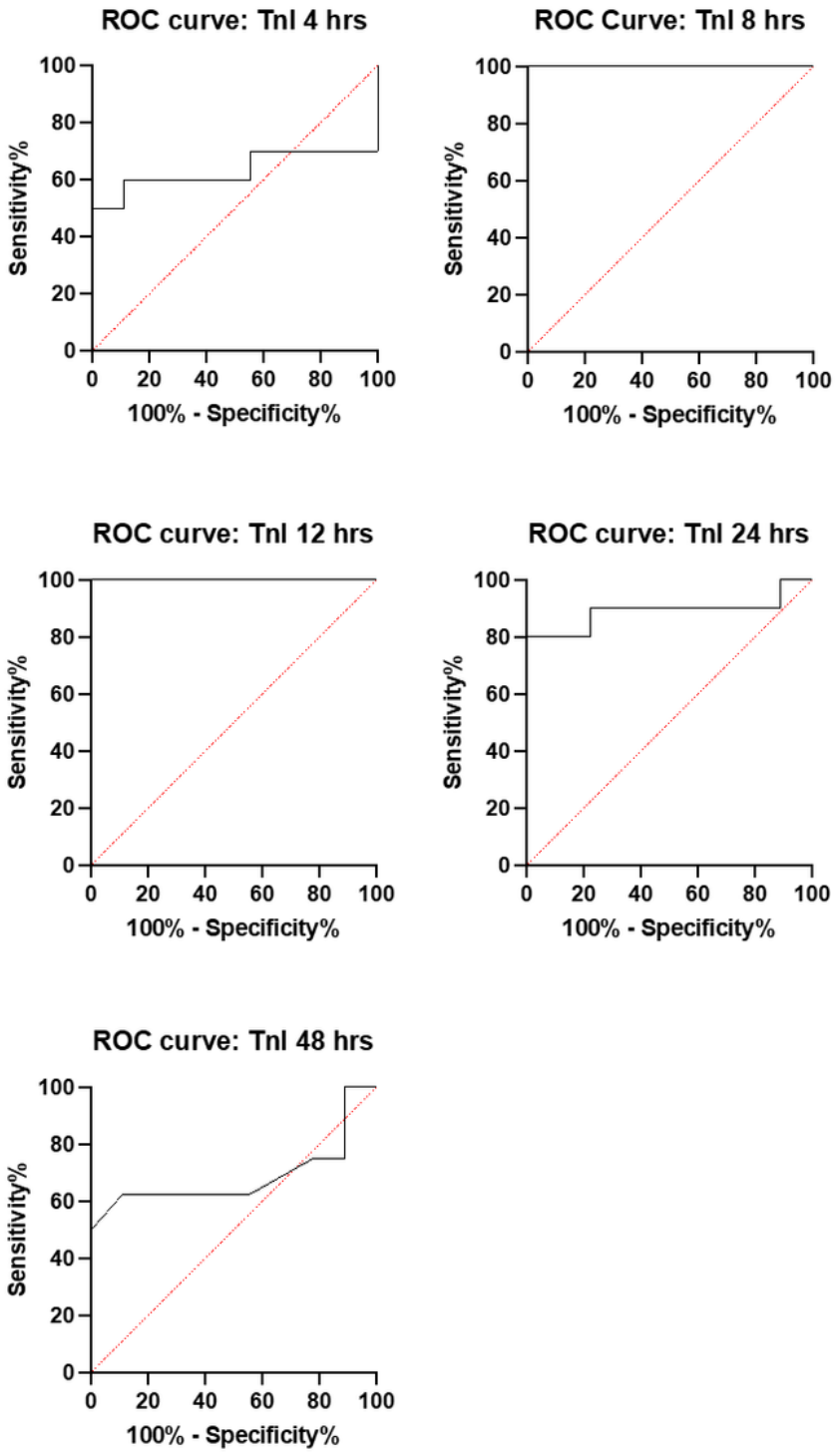

Figure 4

Receiver operating characteristic (ROC) curve for cTnI concentrations discriminates between various time points after the onset of AMI and control group. AUC is calculated to be 0.59,1,1,0.75&0.55 at 4,8,12,24&48 hours respectively.

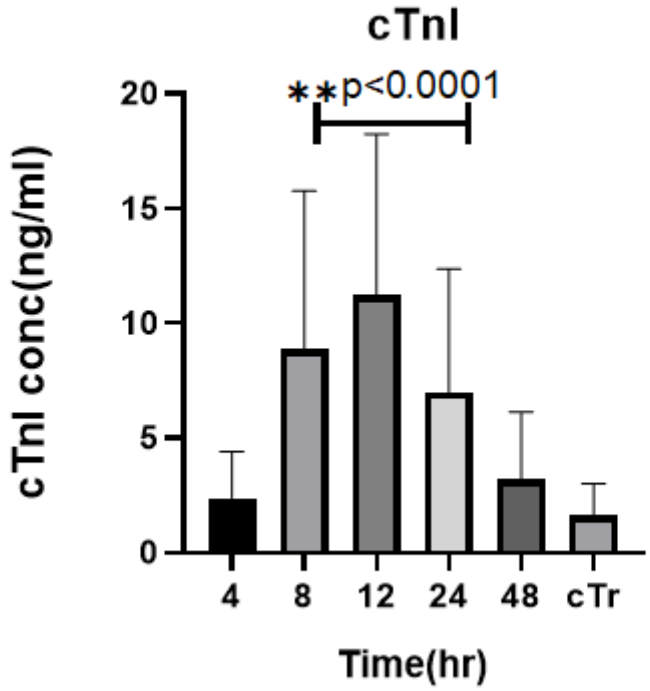

Figure 5

cTnI steady state concentration in ng/ml measured at different time points. Unpaired sample *t*-test was carried for each time point concentration against the control group. * represents $p< 0.05$, ** represents $p<0.01$, *** represents $p< 0.001$.

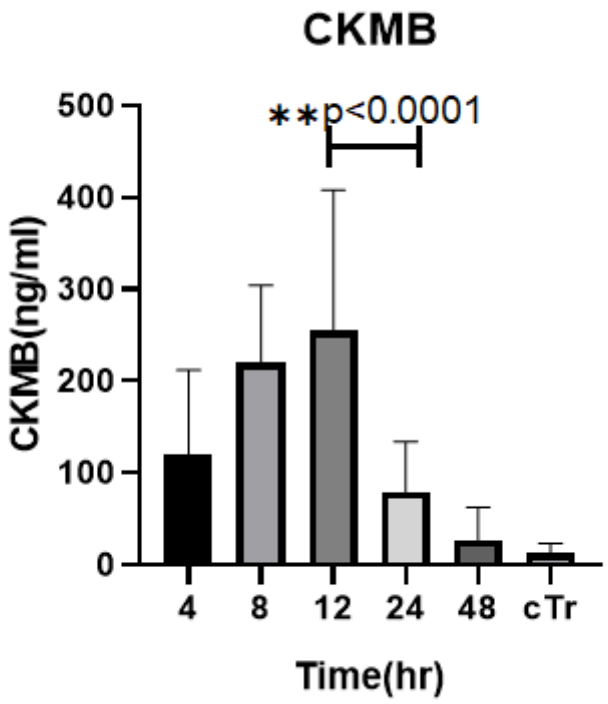

Figure 6

CK-MB steady state concentration in ng/ml measured at different time points. One way ANOVA test, p<0.0001 peak at 12 hours after the onset and decrease after observation. A and B, Scatterplots of plasma CK-MB levels in control subjects versus AMI patients. Within the AMI groups, levels of CK-MB peak showed significantly after 12 hours of MI onset, the same as miR-208b.

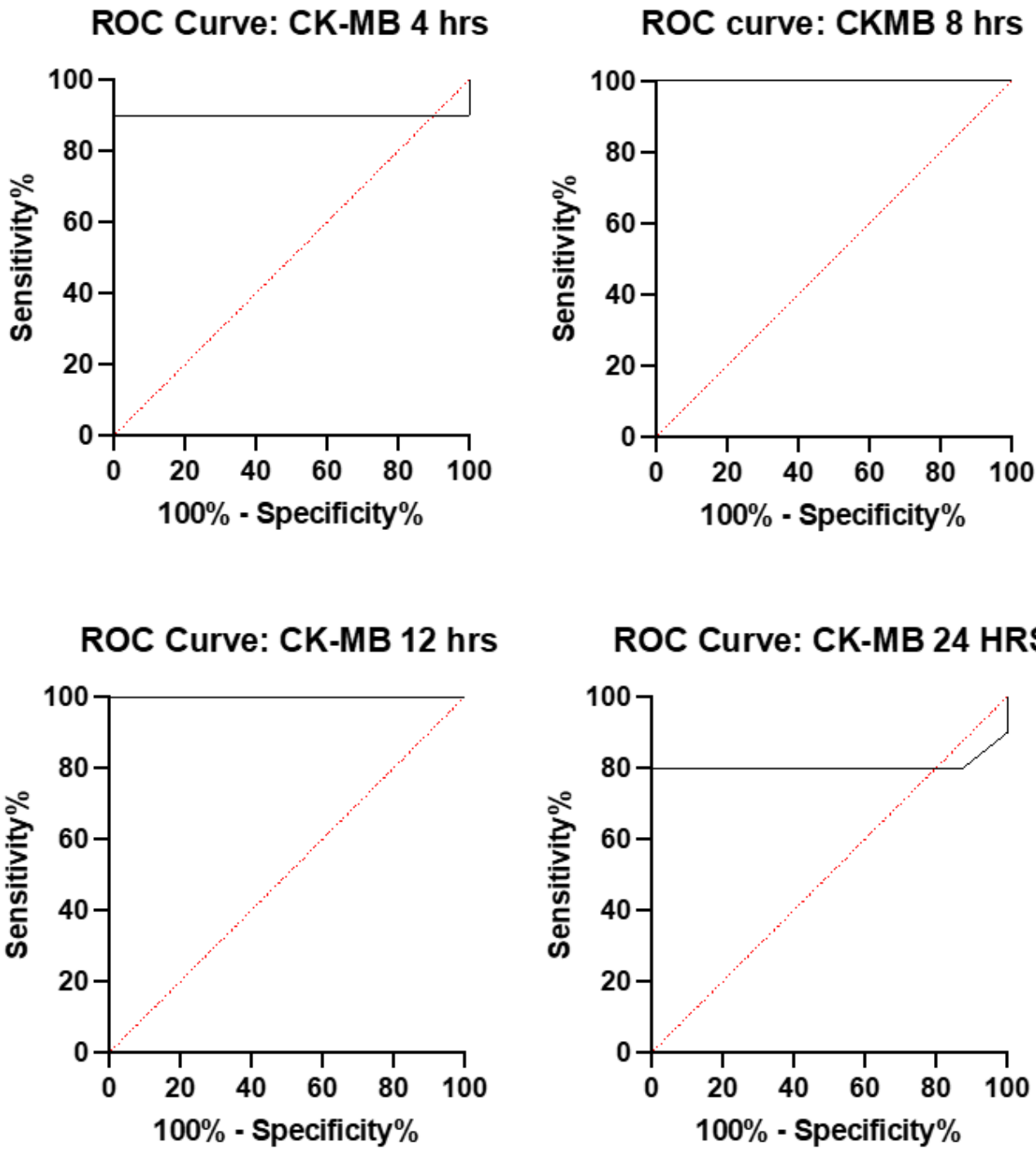

Figure 7

Receiver operating characteristic (ROC) curve for CK-MB concentrations discriminates between various time points after the onset of AMI and control group. AUC is calculated to be 0.59,1,1,0.8&0.73 at 4,8,12,24&48 hours respectively.

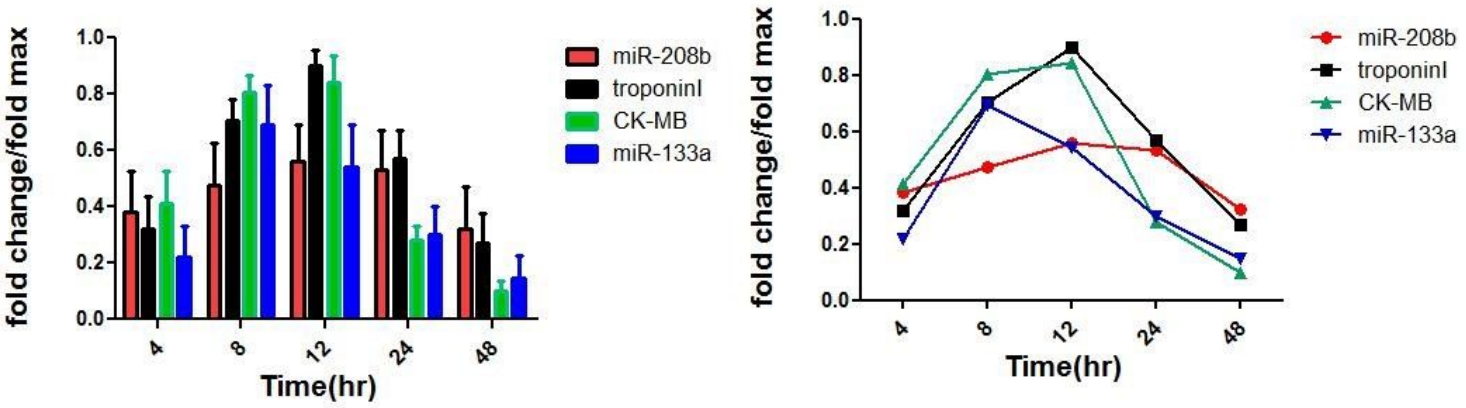

Figure 8

Expression pattern of circulating miR-133a ,208b, cTnI and CK-MB in AMI patients at different time points. Data were normalized to the peak level that miR-133a , 208b, cTnI and CK-MB achieved in each patient ,error bars represent the SEM. On average miRNA 133a achieved a 33.11± 15.59 peak fold change 8 hours after the AMI onset. MiRNA 208b peaks reached at 12 hours achived 50.42±17.60. cTnI and CK-MB, both achieved a peak of 15 ± 2.9 after 12 hours 46.11 ±7.602 12 hours after the same.

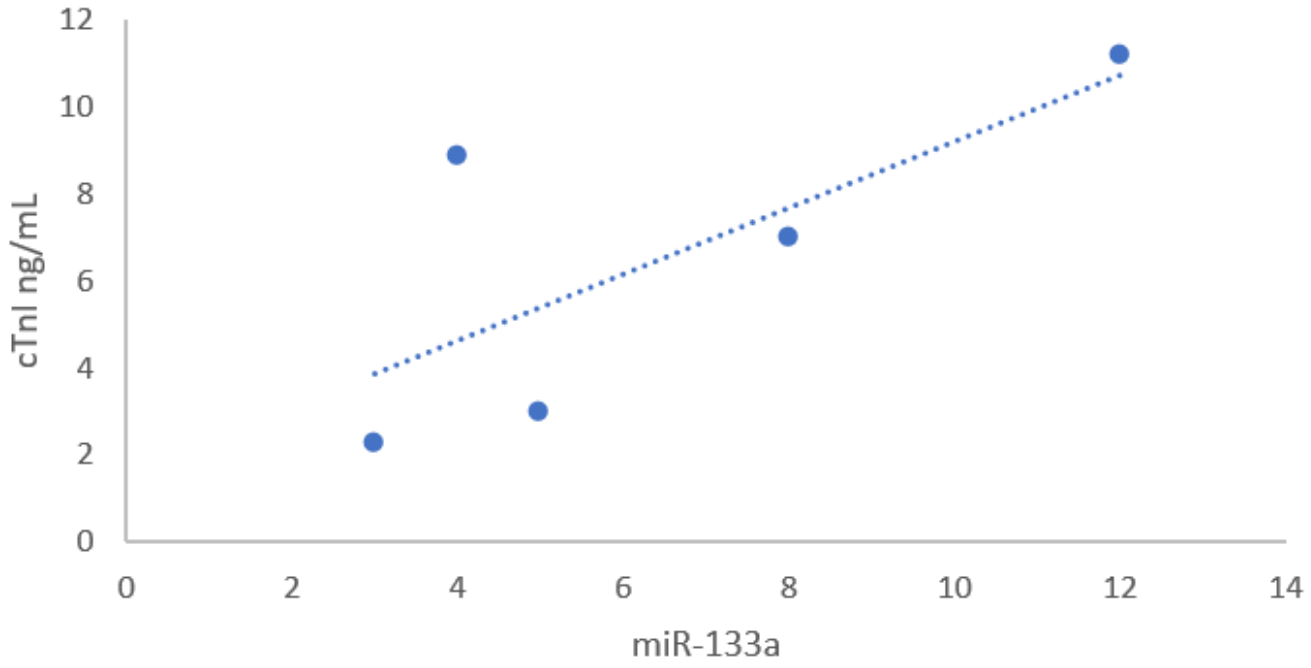

Figure 9

Correlation between miR-133a&cTnI,R =0.926 R^2= 0.858 P =0.0235. Positive correlation between miR-133a and cTnI.

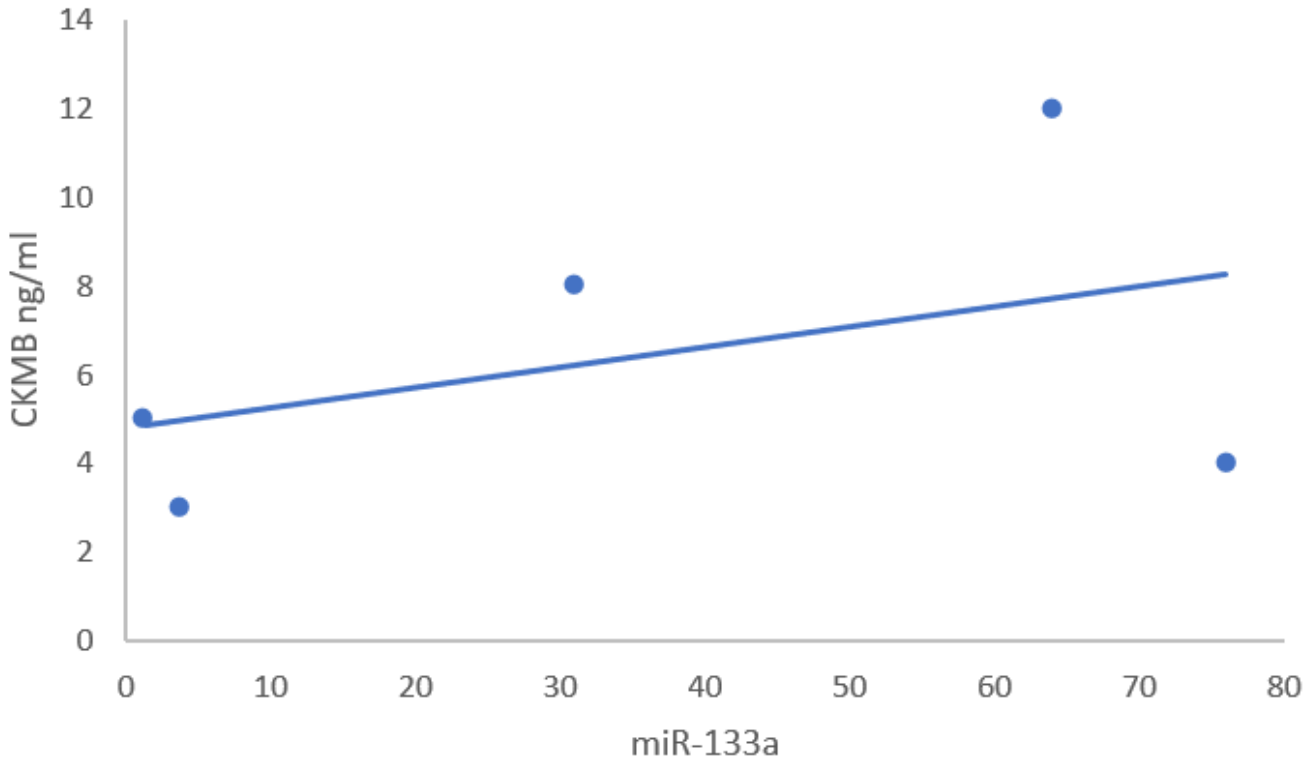

Figure 10

Correlation between miR-133a&CK-MB, R = 0.424 R^2 0.18 P=0.47

No significance correlation between miR-133a and CK-MB

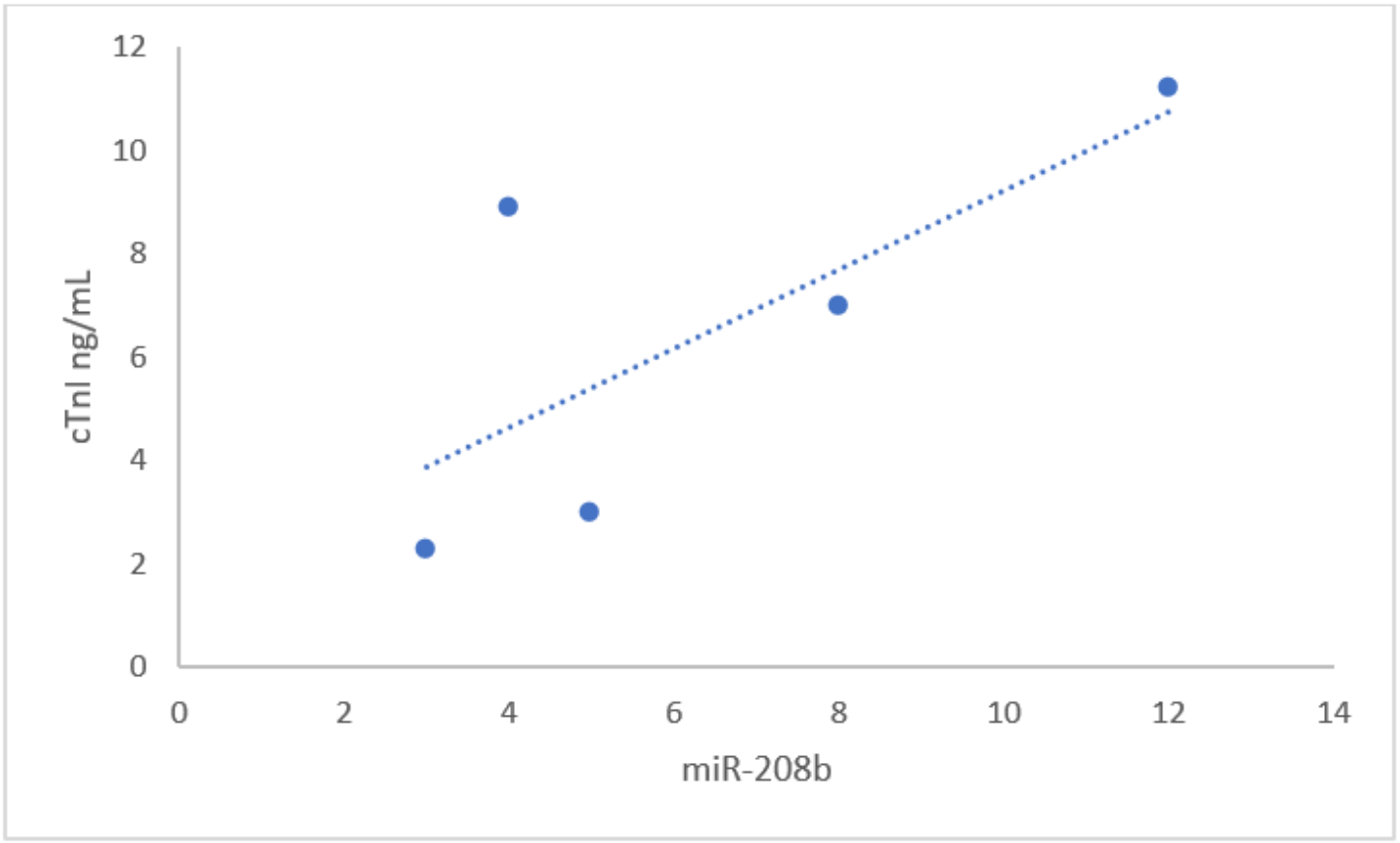

Figure 11

Correlation between miR-208b&cTnI, R=0.8 R^2=0.64 p=0.1

Positive correlation between miR-208b and cTnI.

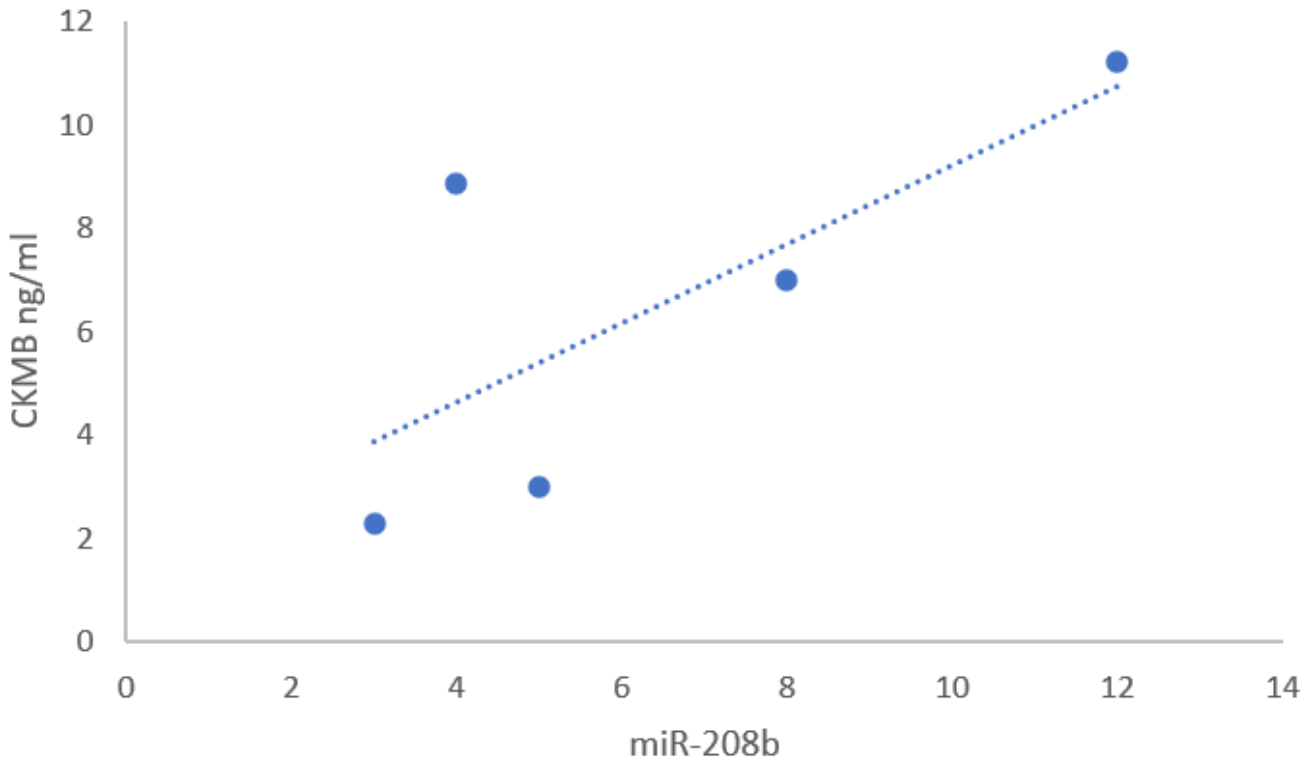

Figure 12

Correlation between miR-208b&CK-MB, R=0.888 R^2=0.789 p=0.044

Positive correlation between miR-208b and CK-MB.

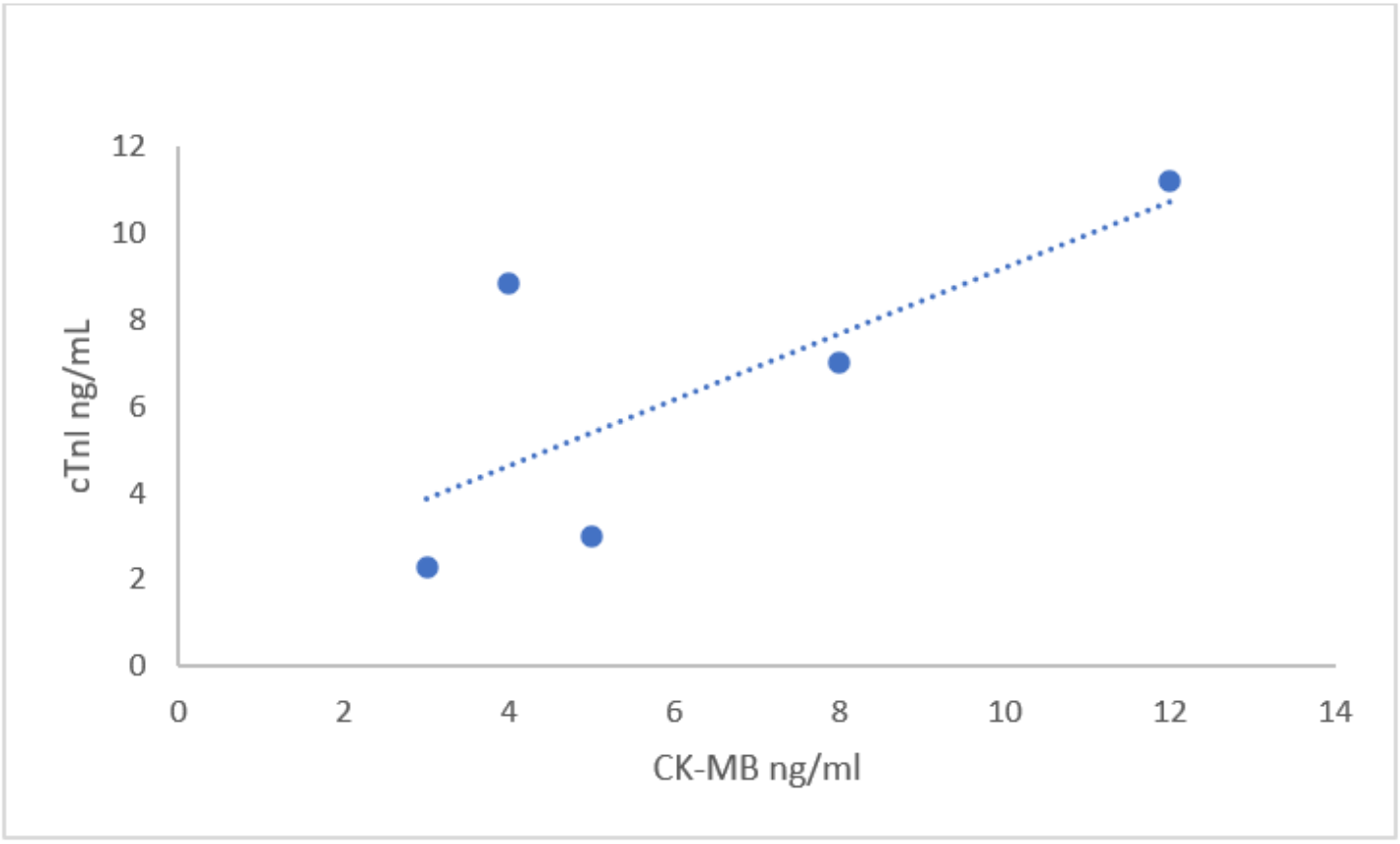

Figure 13

Positive Correlation between CK-MB&cTnI, R= 0.732

Positive correlation between CK-MB and cTnI.