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# DIAGNOSTIC VALUE OFMICRORNA 499 VERSUS HIGH SENSITIVITY TROPONIN IN EARLY DETECTION OF ST-ELEVATION MYOCARDIAL INFARCTION

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**ABSTRACT**

**Background:**Early diagnosis and reperfusion of STEMI patients is crucial to preserve the viability of the ischaemic myocardium, Sowe aimed to prove the diagnostic performance of cardiac enriched microRNA 499 in early diagnosis of STEMI in comparison to high-sensitivity cardiac troponinT (hs-cTnT).

**Subjects and Methods:**Circulating concentrations of cardiac troponin and miR-499 measured byquantitative PCR from 50 Egyptian adult patients presented with STEMI to the emergency department of Menufia University Hospitalin a prospective study conducted during the period from July 2020 to January 2021 then patients population weredivided in two groups,:**Group I:** included 25 patient presented early within the first 4 hour of typical chest pain (TCP), **Group II**: included 25 Egyptian adult patients who presented after 4 hour of TCP,

**Results:**miR-499 highly increased in 50 Patients with STEMI and it has a positive correlation with peak concentrations of cTnT, miR-499 was already detectable in the plasma 1 h after onset of chest pain. In patients who presented within 4 h of onset of pain, miR-499 was positive in 88% of patients and hs-cTnT in only 32%.

**Conclusion:**Circulating miRNAs are powerful markers of acute STEMI,miRNA-499 could be helpfulin early detectionand diagnosis of STEMI in comparison to hs-cTnT.

**Keywords:**STEMI, MiRNA 499, cardiac troponin T.

## INTRODUCTION

STEMI is a leading cause of morbidity and hospitalizations, early reperfusion can reduce the mortality rate of STEMI thus, rapid and accurate diagnosis of STEMI plays a crucial role in therapy and prognosis.*(1)*

Currently, cardiac troponin T (cTnT) is widely used as the most reliable biomarker in clinical diagnosis of AMI and its elevation reflect myocardial necrosis but it can be detected in the plasma 3 hours after onset of chest pain in STEMI patients. However elevated cTnI expression has also been observed in patients with acute, chronic renal failure, heart failure, aortic dissection and other medical conditions. Therefore, we need a more specific serum marker. MicroRNA-499 is a recently discovered member of the microRNA family expressed in myocardium.*(2)*

MicroRNAs (a class of small 19-25 nucleotides noncoding RNAs), are important posttranscriptional regulators of numerous biological processes including cell growth, proliferation, and apoptosis.*(3)*

MiRNA-499 was already detected in the plasma 1 hour after onset of chest pain in STEMI patients, and increased continually and gradually within 9 hours after onset of STEMI.*(4)*

Several studies have suggested a higher diagnostic accuracy of miRNA-499 compared with troponin T for AMI. MiRNA-499 has the advantage of being detectable in the blood within the next four hours after the AMI, whereas troponin can be detected only later. Therefore, miR-499 could enhance the accuracy of troponin T in the early diagnosis of AMI.*(5)*So we aimed to demonstrate that mi-RNA499 can become novel biomarker for early diagnosis of STEMI in comparison to troponin.

## SUBJECTS AND METHODS

The current work was a prospective studyconducted during the period from July 2020 to January 2021 on 50 Egyptian adult patients diagnosed as STEMIpresented at the emergency department of Menoufia University Hospitalandcirculating miR-499 and troponin in serum were assessed at admission, STEMI was defined by clinically significant ST elevation1 mm in any 2 or more contagious leads, ST elevation in V2-V3 >2 mm in old man >40 year or >2.5 mm in young man <40 year or >1.5 mm in woman at any age.*(6)*Then patients were divided in two groups: ***Group1***: 25 patient who presented within the first 4 hour of typical chest pain (TCP),***Group2*:** 25 patients who presented after 4 hour of TCP,

All Patients were subjected to data Full medical historytaking (age, gender, cardiovascular risk factors e.g. hypertension, diabetes mellitus, Dyslipidemia and smoking ) and clinical examination including heart rate, blood pressure measurement and 12-lead ECG within 10 minutes. Then **laboratory work up**: Ten milliliters of Venous blood samples were collected on admission then divided as:

***I- Routine tests including***Cardiac troponin ,creatine kinase-MB fraction.

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***II- Special Investigations***

- Analysis of the expression level of mature miRNA (miR-499) in serum using Taq- Man quantitative reverse-transcription real time PCR**.**Four milliliters were collected on a serum vaccutainer tube, left for 10 minutes to clot andcentrifuged for determination of routine laboratory investigation, two milliliters were collected on a serum vaccutainer tube and centrifuged. The supernatant was stored at -80°C until RNA extraction.**Detection and quantification of miRNAs level were done through the following steps:**

**A- RNA Extraction:** the total RNA -including small RNA- was isolated from serum using QiagenmiRNeasy mini kit which was used for extraction.

**Procedure Steps:**

1. QIAzolLysiswas mixed with serum sample, centrifuged into 3phases:upperphase containing the RNA which transferred to tube; containing 900 µl of 100% ethanol.
2. The mixture was transferred to the miRNeasy Mini spin column and 700 microlitres Buffer RWT were added and centrifuged for 15 s at 40,000.
3. Twenty-five microlitres of RNase-free water were added directly onto the RNeasy Mini spin column membrane, centrifuged for 1 min at 40,000 rpm to elute the miRNA.

**Evaluation of Yield and Quality of RNA, Concentration and Purity** by Spectrophotometerand then was immediately stored at -80°C until its by Quawell Q5000 UV-VIS conversion to cDNA

**B- Reverse transcription (RT):**Reverse transcription (RT) step: cDNA is reversely transcribed from RNA samples using miScript II RT Kit, Mature miRNAs are polyadenylated by poly(A) polymerase and reverse transcribed into cDNA using oligo-dT primers in the same tube allowing amplification of mature miRNA in the real-time PCR step. miScript Primer assays, used in combination with the Taq-Man PCR Kit, enable quantification of mature miRNA by real time PCR

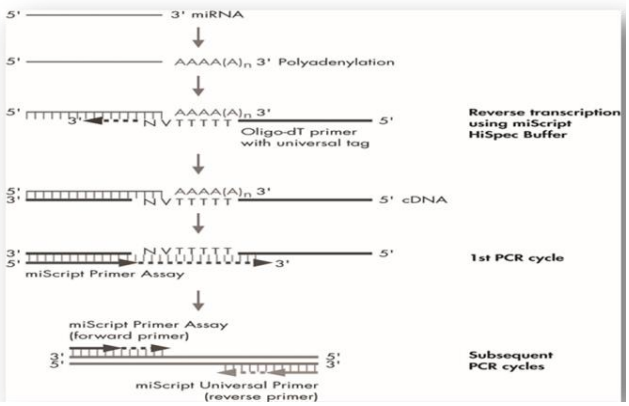


**Fig. (1):** Selective conversion of mature miRNAs into cDNA in miScriptHiSpec Buffer

**C-Real time PCR analysis of circulating miRNAs:** Preparation of PCR reaction plate by transferring the PCR reaction mix into RNase and DNase-free MicroAmp 0.2 ml optical 8-tube strips, the template cDNA was dispensed into RNase and DNase-free MicroAmp 0.2 ml optical 8-tube strips, the tubes were capped and all wells were inspected for uniformity in volume, the plate was loaded into Step One real time PCR system (Applied Biosystems).

**III- Data Analysis using Comparative $C_T$ Method ($\Delta\Delta C_T$)**

**statistical method**

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data with parametric distribution were presented as mean, standard deviations and ranges while with non parametric distribution were presented as median with inter-quartile range (IQR). Also qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using ***Chi-square test***and/or ***Fisher exact test*** when the expected count in any cell found less than 5.

The comparison between two independent groups with quantitative data and parametric distribution was done by using ***Independent t-test*** while with non parametric distribution were done by using ***Mann-Whitney test.***

***Spearman correlation coefficients*** were used to assess the correlation between two quantitative parameters in the same group.

***Receiver operating characteristic curve (ROC)*** was used to assess the best cut off point with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve (AUC).

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

P-value > 0.05: Non significant (NS)

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P-value < 0.05: Significant (S)

P-value < 0.01: Highly significant (HS)

**RESULTS**:

In our studythere was no statistically significant difference between the 2 studied groups as regard age, sex (p=0.750 & 0.684 respectively).As in early group the majority was males (84%) with mean age 53.24 years. In late groupthe majority was male (88%) with mean age 54.20 years as shown in table 1.

**Table (1):**Comparison between early and late groups regarding age and sex.

| | | **Early group** | **Late group** | **Test value** | **P-value** | **Sig.** |
|---|---|---|---|---|---|---|
| | | **No. = 25** | **No. = 25** | | | |
| Age | Mean±SD<br>Range | 53.24 ± 10.81<br>28 – 70 | 54.20 ± 10.34<br>37 – 73 | -0.321• | 0.750 | NS |
| Sex | Female<br>Male | 4 (16.0%)<br>21 (84.0%) | 3 (12.0%)<br>22 (88.0%) | 0.166* | 0.684 | NS |

**P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)**

The main clinical characteristics of the patients showed no statistically significant difference found between early and late groups regarding age and sex and risk factors of the studied cases as shown in table 2.

**Table (2):**Comparison between early and late groups regarding risk factors of the studied patients

| | | **Early group** | | **Late group** | | **Test value*** | **P-value** | **Sig.** |
|---|---|---|---|---|---|---|---|---|
| | | **No.** | **%** | **No.** | **%** | | | |
| DM | No<br>Yes | 10<br>15 | 40.0%<br>60.0% | 16<br>9 | 64.0%<br>36.0% | 2.885 | 0.089 | NS |
| HTN | No<br>Yes | 7<br>18 | 28.0%<br>72.0% | 7<br>18 | 28.0%<br>72.0% | 0.000 | 1.000 | NS |
| Smoking | Non-smoker<br>Smoker<br>Ex-smoker | 13<br>12<br>0 | 52.0%<br>48.0%<br>0.0% | 8<br>16<br>1 | 32.0%<br>64.0%<br>4.0% | 2.762 | 0.251 | NS |
| FH | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 24<br>1 | 96.0%<br>4.0% | 0.000 | 1.000 | NS |
| IHD | No<br>Yes | 21<br>4 | 84.0%<br>16.0% | 18<br>7 | 72.0%<br>28.0% | 1.049 | 0.306 | NS |
| Dyslipidemia | No<br>Yes | 11<br>14 | 44.0%<br>56.0% | 12<br>13 | 48.0%<br>52.0% | 0.081 | 0.777 | NS |
| COPD | No<br>Yes | 23<br>2 | 92.0%<br>8.0% | 25<br>0 | 100.0%<br>0.0% | 2.083 | 0.149 | NS |
| Asthma | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 25<br>0 | 100.0%<br>0.0% | 1.020 | 0.312 | NS |
| ICM | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 25<br>0 | 100.0%<br>0.0% | 1.020 | 0.312 | NS |
| CKD | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 25<br>0 | 100.0%<br>0.0% | 1.020 | 0.312 | NS |
| Obese | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 25<br>0 | 100.0%<br>0.0% | 1.020 | 0.312 | NS |
| Hashish | No<br>Yes | 24<br>1 | 96.0%<br>4.0% | 25<br>0 | 100.0%<br>0.0% | 1.020 | 0.312 | NS |
| Bilateral ll DVT | No<br>Yes | 25<br>0 | 100.0%<br>0.0% | 24<br>1 | 96.0%<br>4.0% | 1.020 | 0.312 | NS |

**DM**: Diabetes Mellitus, **HTN:**Hypertension, **FH:** Family History, **IHD:** Ischemic Heart Dsease, **COPD:** Chronic Obstructive Pulmonary Disease, **ICM:** Ischemic cardiomyopathy, **CKD:** Chronic Kidney Disease**, LL:** Lower Limb, **DVT:** Deep Venous Thrombosis**.**

In all cases there was statistically significant positive correlation found between troponin level and CKMB and micro RNA-499 and there was statistically significant increase in the level of troponin, CKMB and micro RNA-499 levels in late groups than early groups.

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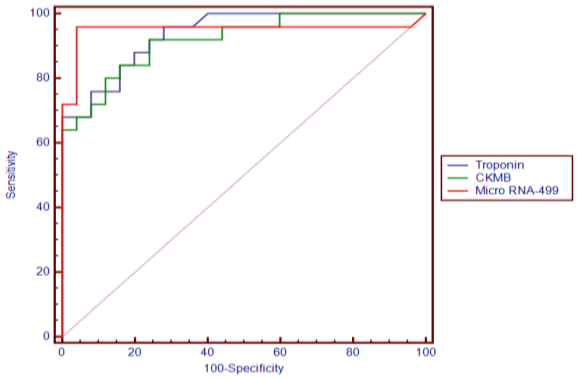
Specifically on Comparison between microRNA-499 and troponin in early group there was significant difference between troponin and MiRNA 499 values as MiRNA 499 was positive in 88%(n 22) and troponin was positive in 32% (n 8) this prove our point of view as shown in table (3)

**Table (3):** Comparison between microRNA-499 and troponin in early group

| | Troponin | | Micro RNA | | Test value* | P-value | Sig. |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | | | |
| Negative | 17 | 68.0% | 3 | 12.0% | 16.333 | 0.000 | HS |
| Positive | 8 | 32.0% | 22 | 88.0% | | | |

The ROC curve in our study shows that the best cut off point for micro RNA-499 to differentiate between early and late groups was found > 3.1 with sensitivity of 96.0%, specificity of 96.0% and area under curve (AUC) of 95.1% while for troponin level the best cut off point was found > 2.1 with sensitivity of 92.0%, specificity of 76.0% and AUC of 93.7%. Lastly the best cut off point for CKMB which was found > 35 with sensitivity of 92.0%, specificity of 76.0% and AUC of 91.8%.




| Parameter | AUC | Cut of Point | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| **Troponin** | **0.937** | **>2.1** | **92.0** | **76.0** | **79.3** | **90.5** |
| **CKMB** | **0.918** | **>35** | **92.0** | **76.0** | **79.3** | **90.5** |
| **Micro RNA-499** | **0.951** | **>3.1** | **96.0** | **96.0** | **96.0** | **96.0** |

**Fig.(2):**Receiver operating characteristic curve (ROC) for troponin, CKMB andmicro RNA-499 levels in the 2 groups

**DISCUSSION**

STEMI is the most acute manifestation of CAD, with substantial morbidity and mortality, early reperfusion preserve the viability of the ischaemic myocardium and limit infarct size so early diagnosis of STEMI is crucial to initiate appropriate treatment, STEMI is diagnosed by ST segment elevation >1 mm in any 2 or more contagious leads, ST elevation in V2-V3 >2 mm in old man >40 year or >2.5 mm in young man <40 year or >1.5 mm in woman at any age.*(6)*

Blood tests for biomarkers of myocardial injury are indicated as soon as possible in the acute phase, but reperfusion treatment should not be delayed waiting the results, among these biomarkers CK, CKMB, cardiac troponins and myoglobin, recent studies have revealed micro RNA 499 as a new biomarker in early diagnosis of STEMI.*(7)*

The use of troponin as biomarker for detection of myocardial ischaemia has undergone rapid evolution;troponin level is detectable 3h after myocardial infarction, and remained above normal for up to 7–10 days and may be detected in blood after 14 day.*(8)*

MiRNAs are small (18–24 nucleotides) non-coding single-stranded RNA molecules that help to fine-tune gene expression,miR-499 is a member of the microRNA family encoded by myosin, it regulates the expression of the beta myosin heavy chain, resulting in the enhancement of myocardial oxygen metabolism and tolerance, it has also been demonstrated that the expression levels of miR-499 exhibit a significant change in certain heart diseases, including AMI.*(9)*

The gene encoded miR-499 is embedded within a ventricular specific myosin heavy chain gene, which is almost exclusively expressed in the heart, plasma level of miR-499 was obviously increased in all patients with AMI. In AMI patients with chest pain < 3 hour, miR-499 was positive in 93% of patients while the positive rate for cTnT was 88%, suggesting that miR-499 is a sensitive and novel biomarker for the diagnosis of AMI.*(10)*

The aim of our study was to compare the clinical applicance of microrna 499 as a new laboratory parameter versus troponin in early diagnosis of STEMI, it was conducted on 50 patients divided into two groups, the first group

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included 25 patients presented early within 4 hours of typical chest pain onset and the second group included 25 patients presented late after 4 hours of typical chest pain onset.

The two groups were subjected to informed consent, full medical history,cardiovascular risk factors (e.g. hypertension, diabetes mellitus, smoking and their duration, typical chest pain duration on admission, current medications and family history) and full clinical examination including general condition, heart rate, blood pressure measurement, standard twelve lead ECG recording and interpretation were obtained at the point of FMC with a maximum target delay of 10 minutes.

Routine tests including cardiac troponin (cTn), serum creatine kinase-MB fraction (CK-MB), complete blood count (CBC) were done, analysis of the expression level of mature miRNA (miR-499) in serum was done using Taq- Man quantitative reverse-transcription real time PCR.

In early group the median value of troponin, microRNA-499 was (0.33,1.5 respectively), while in late group the median value of troponin, microRNA-499 was (6.5, 9.5 respectively).

Our study showed that microRNA has higher sensitivity and specificty in comparison to troponin and CKMB among all patients where sensitivity of microrna, troponin and CKMB is (96%, 92%, 92%), respectively and specificity of microrna, troponin and CKMB is (96%, 76%, 76%) respectively, especially in early group, our study showed positive result of troponin and microrna 499 in 32% and 88% of patients respectively and negative result of troponin and microrna 499 in 68% and 12% of patients respectively indicating that microrna 499 has higher sensitivity and specificity in early detection of STEMI, this prove that microRNA 499 increase early in cases of STEMI and can be detected in blood within the 1 hour from TCP sensation so it can be used in early diagnosis of ACS.

This agrees with the results of ***Yao et al., 2014*** and ***Devaux et al., 2015***who found that the miRs values increased rapidly and reached a peak at 1–3 h whereas cTnI peaked later at 6 h Thus, miRs have an earlier time course than the traditional protein biomarker troponin.*(11,12)*

On the other side ***Xin et al., 2016*** demonstrated the difficulty of use of miR-499 in clinical practice as, miR-499 can only be detected by PCR, which is time and labor consuming, and is not facilitated in early diagnosis of AMI.*(4)*

**CONCLUSION**

MiRNA 499 has higher sensitivity and specificity in early detection of STEMI in comparison with troponin and it can be used as a new marker for early detection of STEMI.

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