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Determining the level of miRNA 133a-5p and Mid Regional pro-Adrenomedullin in Patients with Stable Angina Pectoris

Aseel Ghassan Daoud[*, 1], Ahmed Yousif Hasan[2] and Wassan Abdul Kareem Abbas[1]

[1] Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq
[2] Ministry of Health , Iraqi Center for Heart Diseases, Al-Shaheed Ghazi Al-Hareery Hospital, Baghdad, Iraq

[*]**Corresponding author**
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Abstract

Chronic stable angina pectoris (CSAP) is a prevalent heart disease which can be defined as a symptom or a clinical syndrome of stable coronary artery disease (CAD). CAD is usually caused by the presence of atherosclerotic plaques or blood clot within coronary arteries that may lead to ischemia. miRNA 133a is mainly expressed in the muscle and it has a role in apoptosis, angiogenesis, hypertrophy, atherosclerosis and ischemia. Mid regional pro-Adrenomedullin (MR-proADM) is a member of calcitonin peptide family and adrenomedullin (ADM) precursor but with a longer plasma half-life and more physiologically stable than adrenomedullin (ADM), of endothelial origin and produced by different sites in the body mainly by the heart, kidneys, lungs and adrenal medulla. MR-proADM plasma level is thought to be predictive of CAD. The aim of the current study was to detect the serum levels of microRNA 133a-5p and MR-proADM in a sample of Iraqi patients with chronic stable angina and obstructive CAD (since there are many causes to have CSAP and one of them is CAD) with normal left ventricular ejection fraction (LVEF) so that to investigate whether these biomarkers can be used to diagnose those patients. In this case-control study, fasting venous blood samples (this is the protocol, the patient should be fasting because during catheterization, the patient might get some complications like perforation, dissection, or unstable left main stem (LMS) artery, so the patient should be referred for surgery urgently) were collected from 90 male and female (the reference serum expression of microRNA 133a are low in both sexes and the reference serum level of MR-proADM is usually similar in both sexes) subjects (40-76 years old) who were presented to the outpatient clinic as having chest pain (the blood samples were collected from the patients after referring them to the Iraqi center for heart diseases not at the time of their visit to clinic, so they were instructed to come fasting at the day of their angiography at the center). An electrocardiography, echocardiography and conventional coronary angiography were applied by the cardiologist for all the participants. Routine biochemical tests and patients questionnaire were also applied. Left ventricular ejection fraction was detected using the biplane M mode method. Accordingly, the patients who showed evidence of atherosclerotic plaque and CAD on the angiography were classified as angina patients (n=60) and those who showed no evidence of coronary obstruction and ischemia on the angiography (normal angiography) were classified as being a control group (n=30). The fold of gene expression of miRNA 133a-5p in serum and serum levels of MR-proADM, fasting blood glucose (FBG), urea, creatinine, TGs, total cholesterol, LDL-C, HDL-C and VLDL-C were measured. Results: the fold of miRNA 133a-5p gene expression in serum and the serum levels of MR-proADM were significantly higher in patients ($P \leq 0.01$) and ROC curve showed that miRNA 133a-5p had 98% sensitivity and 47% specificity with AUC=0.8, whereas, MR-proADM had 100% sensitivity and 100% specificity with AUC=1.0. So, it can be concluded that MR-proADM could be used as an independent biomarker for the diagnosis of stable angina patients with normal LV ejection fraction, whereas microRNA 133a-5p is better to be used in combination with other biomarkers to give more accurate diagnosis.

Keywords: Angina, Atherosclerosis, Diagnosis, miRNA 133a-5p, MR-proADM.

Introduction

Myocardial ischemia represents one of the major causes of death from heart diseases all around the world which occurs as a result of myocardial demand and oxygen supply imbalance [(1,2)].

It can be classified into chronic coronary syndromes and acute coronary syndromes [(3)]. It may occur as a result of multiple factors that lead to the stenosis or occlusion of one or more of the coronary

arteries like: atherosclerotic plaque, coronary microvascular dysfunction and coronary vasospasm [4].Coronary arteries occlusion usually occurs as a result of blood clotting or atherosclerotic plaque within these arteries leading to myocardial ischemia [5,6]. There are many risk factors that increases the incidence of CAD some of which are non-modifiable like: age, gender, family history and ethnicity, and some others are modifiable like: dyslipidemia, hypertension, DM and smoking [7–10]. The first step in the diagnosis of CSAP is the physical examination of the patients and their history regarding the severity, type and duration of the pain, since those patients will usually experience a squeezing chest pain occurs on exertion and relieved at rest. Also taking the medical history of the patient, assessing the blood pressure, measuring the body weight, cigarette smoking, alcoholic or not and exercise tolerability [11]. There are also many techniques which are used for the diagnosis like: Pre-Test Probability, Coronary Computed Tomography Angiography, Stress echocardiography, Stress electrocardiogram (ECG) and Invasive Coronary Angiography [12–16].

Micro-RNAs are small non-coding single stranded RNA molecules of about 20-22 nucleotide in length that regulate the gene expression of messenger RNA (mRNA) via negative post transcription, hence controlling different cellular processes in the body [17–19]. There is a number of miRNAs which are released by different sites of the heart and its level has thought to be changed in different cardiovascular diseases like cardiac hypertrophy, heart failure, myocardial infarction and arrythemia [20]. From these miRNAs, miRNA 133a is mainly expressed in the cardiac and skeletal muscle and it has a role in apoptosis, angiogenesis, hypertrophy and cardiac fibrosis [20,21]. miRNA 133a is considered to be one of the cardiac miRNAs (miR-1, miR133a, miR-208a/b, and miR-499) since it is expressed abundantly in the myocardium and involved in early stage of cardiogenesis so it has cardiac-specific muscle lineage [22]. It also plays a role in atherosclerosis progression and ischemia by its effect on smooth muscle proliferation, endothelial injury and macrophage activity [23].

Mid regional pro-Adrenomedullin (MR-proADM) is a member of calcitonin peptide family and adrenomedullin (ADM) precursor but with a longer plasma half-life and more physiologically stable than ADM. MR-proADM is of endothelial origin and produced by different sites in the body mainly by the heart, kidneys, lungs and adrenal medulla. Its blood level is elevated in many disease conditions like renal failure, heart failure and ischemic heart diseases especially myocardial infarction [24]. MR-proADM plasma level is thought to be predictive of CAD [25]. It plays a role in the estimation of left ventricular ejection fraction in CAD patients [26]. The aim of the current study was to detect the serum levels of microRNA 133a-5p and MR-proADM in a sample of Iraqi patients with chronic stable angina and obstructive CAD with normal LV ejection fraction so that to investigate whether these biomarkers can be used to diagnose those patients.

Materials and Methods

Patients:

This case-control study was approved by the local ethical committee of the College of Pharmacy/Mustansiriyah University. It was performed on 90 participants who were presented to the outpatient clinic as having chest pain, all were asked to fill signed informed consents. Helsinki statement by The World Medical Association (WMA) was applied in this study. The samples were collected from the Iraqi center for heart diseases from Al-Shaheed Ghazi Al-Hareery/ Medical City teaching hospital at the period from November 2022 to April 2023.The subjects were examined by echocardiogram which was performed by the cardiologist at his clinic to detect any regional wall motion abnormality and for any ischemic changes, then accordingly they were referred for coronary angiography by the cardiologist to check the coronary arteries.

The severity of ischemia and the degree of coronary arteries stenosis was assessed according to American Heart Association [27] (stenosis > 70% was considered critical, stenosis 50-69% was considered intermediate and stenosis < 50 was considered low risk), as well as the number of the diseased vessels: single vessel, two vessels or three vessels. So depending on the result of the angiography, those subjects were classified into two study groups: angina patients (n=60) and control (n=30). The subjects who showed no evidence of CAD or ischemia on coronary angiography were enrolled in the control group.

Inclusion criteria:

Male and female patients with chronic angina who presented with chest pain with or without ECG changes reflecting ischemia, age of 40-76 years old, left ventricular ejection fraction LVEF ≥ 50%, no or controlled hypertension and diabetes mellitus.

Exclusion criteria:

Patients with myocardial infarction, renal failure or chronic kidney disease, valvular heart disease, congenital heart disease, stroke, chronic heart failure, atrial fibrillation, malignancy and immune diseases.

Methods:

Sample collection:

About 6 ml of fasting venous blood samples were collected from each subject before the coronary angiography session had been performed. The blood samples were centrifuged at 3000 rpm for 10 minutes to isolate serum, which was divided into eppendorf tubes and stored at – 40 c^{o} till the time of

the assessment. About 500 µl of the isolated serum from each subject were placed in PCR tubes contained 500 µl of TRIzol for the later extraction of microRNA 133a-5p.

Echocardiography and electrocardiography

All patients were subjected to echocardiography and ECG with LV ejection fraction was calculated using the biplane M mode method.

Coronary angiography

All the patients were referred for coronary angiography at the Iraqi center for heart diseases/Al-Shaheed Ghazi Al-Hareery/ Medical City Teaching Hospital to be performed by the cardiologist himself and his team using X-ray conventional coronary angiography which was applied by inserting a catheter with a contrast dye into the femoral or radial artery under local anesthesia. The degree of stenosis and the number of the diseased vessels were determined. All of them were instructed to be fasting for at least 8 hours before the procedure (this is the protocol, the patient should be fasting because during catheterization, the patient might get some complications like perforation, dissection, or unstable left main stem (LMS) artery, so the patient should be referred for surgery urgently). Loading doses of the antiplatelet medications, aspirin 300 mg and clopidogrel 600 mg tablets, were prescribed to be taken prior the procedure with the unfractionated heparin as an anticoagulant was injected during the procedure.

Assay of the studied biomarkers

MicroRNA 133a-5p

The primers were synthesized and lyophilized by Alpha DNA Ltd. (Canada) [28–30]. For the gene expression steps included: total RNA extraction and microRNA purification was done by using EasyPure® miRNA Kit/ TransGen biotech./China following the manufacturer's instructions, then Complementary DNA (cDNA) synthesis from miRNA by the reverse transcription of the extracted miRNA using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit/TransGen biotech./China following the manufacturer's instructions, and finally, the qRT-PCR SYBR Green test was used to estimate the expression level of miRNA 133a-5p. U6 miRNA was used as a housekeeping endogenous gene against miRNA 133a-5p gene. The TransStart® Top Green qPCR Super Mix kit/ TransGen biotech./China was used to measure the threshold cycle (Ct) and to estimate the fold changes and the expression levels of miRNA 133a-5p and miRNA U6 genes in which every reaction was done twice. The Quantitative Real Time PCR (qRT–PCR) was performed using the QIAGEN Rotor gene Q Real-time PCR System (Germany). The double delta Ct ($2^{-\Delta\Delta Ct}$) analysis was used to calculate fold changes of the quantified gene expression.

MR-proADM

Its serum level was measured using Human MR-ProADM Sandwich ELISA Kit / Elabscience/ USA.

Other biomarkers measured:

Serum creatinine, urea, fasting blood glucose, total cholesterol, HDL-C and triglycerides were assessed using kits of Selectra pro M biochemistry analyzer/ ELITech/ France. Serum levels of LDL-C and VLDL-C were estimated using Friedewald equation.

The Statistical Analysis:

To detect the effect of different factors on the studied parameters, IBM SPSS Statistics 26 program was used for the statistical analysis. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability). One-way ANOVA and T-test was used to significantly compare between means. GraphPad Prism 9 program was used to draw the figures in this study [31,32].

Results and Discussion

Demographic distribution data and clinical characteristics between the patients and the control groups

The patients and the control groups were presented by their age, gender, body mass index (BMI), family history, smoking status and left ventricular ejection fraction (LVEF), in addition to the medical and the medication history of the patients included in the study regarding dyslipidemia, hypertension, diabetes mellitus, statins and beta blockers, as illustrated in Table 1. There were no significant differences between the patients and the control regarding the age distribution, BMI, family history, smoking status and LVEF ($P>0.05$).

On the other hand, there was a high significant difference regarding gender distribution between the patients and the control groups ($P\leq0.01$). However, the higher percentage of patients were males (78%) whereas females were only (22%). It was found that 52% of patients had dyslipidemia while 48% of them did not with a highly significant difference between them ($P\leq0.01$). Besides, the current study also detected a high significant difference between the patients who had hypertension (65%) and those who did not have hypertension (35%) with ($P\leq0.01$). Also, It was detected that (46.7%) of the patients had DM while (53.3%) did not have DM with a high significant difference ($P\leq0.01$). In addition the present study detected no significant difference between patients who were on statins (50%) and those who were not (50%) ($P>0.05$), while there was a high significant difference between patients who were on β-blockers (33%) and those who were not (67%) ($P\leq0.01$).

Table 1. Demographic distribution data and the clinical characteristics of the patients and the control groups.

Characteristics	CAD negative (no.=30)	CAD positive (no.=60)	*P*- value
Age (years) Mean ± SD	58.53 ± 9.40	59.35 ± 9.32	0.6
BMI (kg/m^2) Mean ± SD	28.67 ± 3.97	28.35 ± 4.11	0.7
Gender Male n (%) Female n (%) Total	 12 (40%) 18 (60%) 30 (100%)	 47 (78%) 13 (22%) 60 (100%)	0.001
Family history of heart disease yes No Total	 12 (40%) 18 (60%) 30 (100%)	 25 (42%) 35 (58%) 60 (100%)	0.8
Smoking Yes No Total	 6 (20%) 24 (80%) 30 (100%)	 9 (15%) 51 (85%) 60 (100%)	0.5
Dyslipidemia Yes No *P*-value	 _	 31 (52%) 29 (48%) 0.001	 _
Hypertension Yes No *P*-value	 _	 39 (65%) 21 (35%) 0.001	 _
DM Yes No *P*-value	 _	 28 (46.7%) 32(53.3%) 0.001	 _
LVEF (%) Mean ± SD	64.07 ± 6.51	63.42 ± 7.24	0.6
Statins Yes No *P*-value	 _	 ٣٠ (٥٠%) ٣٠ (٥٠%) 1	 _
Beta blockers Yes No *P*-value	 _	 20 (33%) 40 (67%) 0.001	 _

The data were expressed as Mean ± SD, Chi-square test was used to significantly compare between percentages ,Significant difference ($P \leq 0.05$), Highly Significant difference ($P \leq 0.01$), no significant difference ($P > 0.05$), BMI: Body Mass Index, no.: number, % percentage, SD: standard deviation, DM: diabetes mellitus, LVEF: left ventricular ejection fraction.

Comparison in the gene expression level of serum micro-RNA 133a-5p between the patients and the control groups

The gene expression fold of *miR 133a-5p* was measured against the housekeeping reference gene *miR U6* based on ΔCt method ($2^{-\Delta Ct}$). The ΔΔCt ($2^{-\Delta\Delta Ct}$) was also used. It was found that the fold of expression in the serum of patients (2.03) was highly significant compared to that in the control group (1.00) and ($P \leq 0.01$). The folds, amplification plot and melt curve of *miR 133a-5p* gene are shown in Figures (1-A), (1-B), and (1-C) respectively.

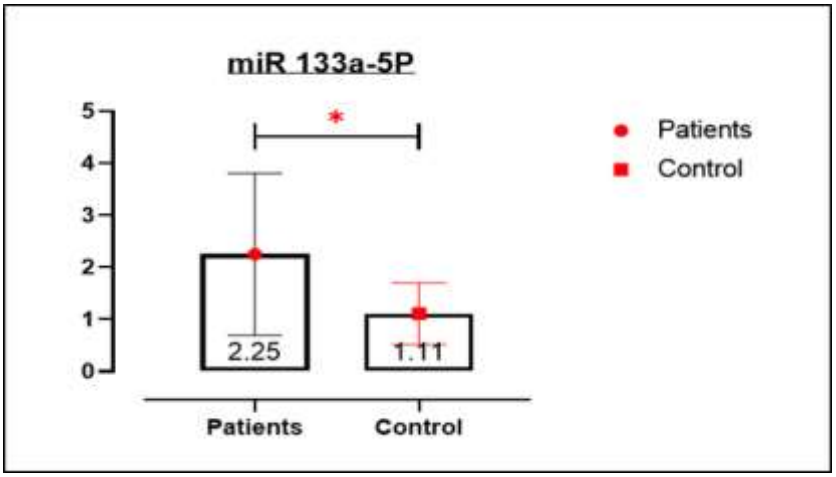

Figure (1-A). Folds of miRNA 133a-5p gene expression between the patients and control

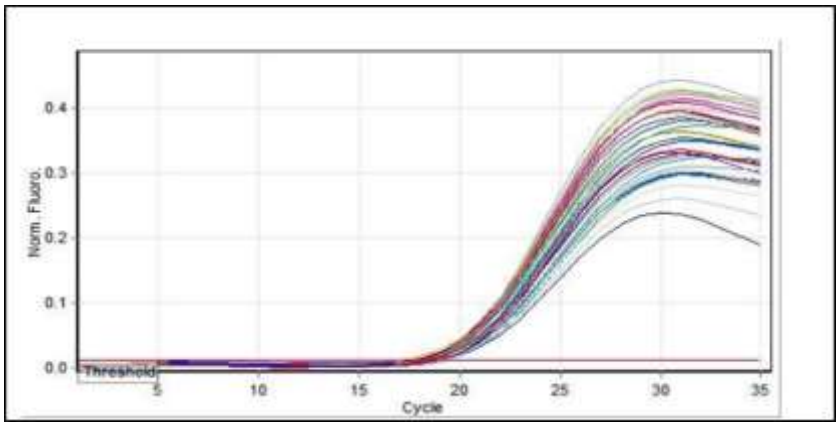

Figure (1-B). The amplification plot of *miR133a-5p* gene by RT-PCR. The picture was taken directly from the device.

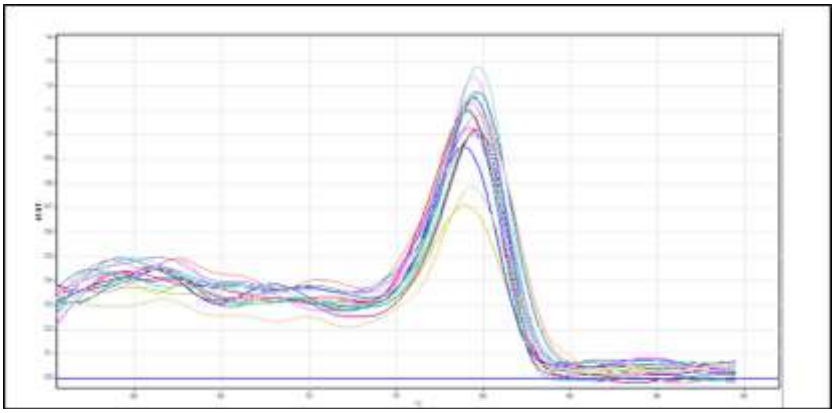

Figure (1-C). Melt curve of *miR133a-5p* gene amplicons describing the peak following analysis by RT-qPCR.

Comparison in the serum levels of mid-regional pro Adrenomedullin and other studied biomarkers between the study groups

The results were clarified in Table 2. The present study found highly significant increases in the serum levels of MR-proADM in the patients compared with the control group, ($P \leq 0.01$) as shown in Table 2 and Figure.2. Regarding serum total cholesterol, HDL-C, LDL-C, urea and FBG, there were no significant differences between the patients and the control groups ($P > 0.05$). On the other hand, there was a significant difference in serum TGs and VLDL-C between the patients and the control groups ($P \leq 0.05$). There was a high significant difference in the serum creatinine level between the patients and control groups.

Table 2. Comparison in the serum levels of MR-proADM and other studied biomarkers between the study groups:

Biomarker	Mean±SD		*P*-value
	CAD negative (no.=30)	CAD positive (no.=60)	
MR-proADM (pg/ml)	181.91 ± 8.50	335.11 ± 71.35	0.001
Total cholesterol (mg/dl)	143.96±32.27	148.73±48.06	0.6
TGs (mg/dl)	143.46±76.73	185.50±100.88	0.04
HDL-C (mg/dl)	40.47±11.18	36.70±13.15	0.1
LDL-C (mg/dl)	74.83±33.40	75.01±40.85	0.9
VLDL-C (mg/dl)	28.87±15.14	37.10±20.18	0.05
S.Cr. (mg/dl)	0.76±0.19	0.89±0.20	0.004
Urea (mg/dl)	32.11±7.42	33.61±7.27	0.3
FBG (mg/dl)	105.36±25.72	132.78±77.10	0.06

The data were expressed as Mean ± SD; T-test was used to significantly compare between means; Significant difference (P≤0.05), Highly Significant difference (P≤0.01), no significant difference (P>0.05); MR-proADM: mid-regional pro adrenomedullin; TGs: triglycerides; HDL-C: high density lipoprotein- cholesterol; LDL-C: low density lipoprotein-cholesterol; VLDL-C: very low density lipoprotein-cholesterol; S.cr.: serum creatinine; FBG: fasting blood glucose.

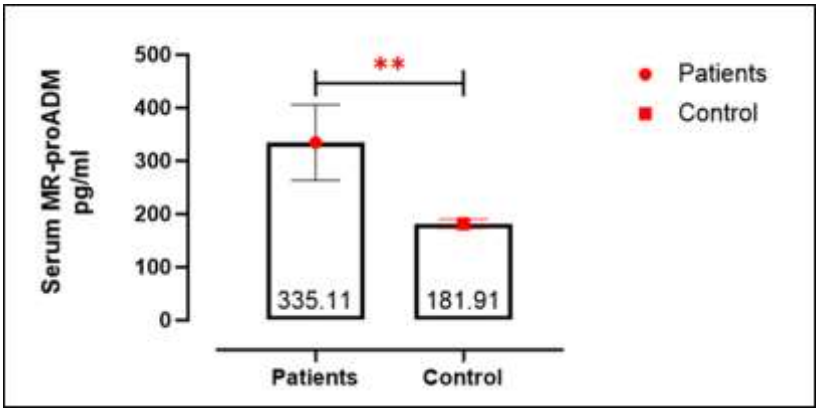

Figure 2. The difference in the serum level of MR-proADM between the patients and the control groups.

The effect of the number of diseased coronary vessels on the serum levels of the studied biomarkers

The effect of the number of occluded coronary arteries on the serum levels of the studied biomarkers are summarized in Table 3. The current study detected a significant difference in the fold of miR133a-5p gene expression in patients with single vessel compared to those patients who had two-vessel and three-vessel disease (*P*≤0.05). On the other hand, there were no significant differences detected among the patients groups regarding serum levels of MR-proADM, total Cholesterol, HDL-C, TGs, LDL-C, VLDL-C, urea, creatinine and FBG where (*P*>0.05).

Table 3. Level of different biomarkers in patients with different number of diseased coronary vessels:

Biomarker	Mean±SD			*P*-value
	Patients SVD (no.=12)	Patients 2VD (no.=23)	Patients 3VD (no.=25)	
MR-proADM (pg/ml)	350.18±82.75	334.50±64.53	328.44±73.49	0.6
Fold of *miR133a-5p* gene expression	3.05 a±2.10	1.75 b±0.87	2.33 b±1.64	0.05
T.Cholesterol (mg/dl)	155.08±49.16	144.00±52.35	150.04±44.89	0.8
HDL-C (mg/dl)	40.75±12.33	34.65±11.78	36.64±14.70	0.4
TG (mg/dl)	190.42±78.94	188.96±104.01	179.96±110.40	0.9
LDL-C (mg/dl)	76.51±44.70	71.63±48.76	77.40±31.46	0.8
VLDL-C (mg/dl)	38.09±15.82	37.80±20.79	35.98±22.08	0.9
Urea (mg/dl)	35.00±6.95	33.48±8.20	33.06±6.69	0.7
S.cr. (mg/dl)	0.90±0.17	0.87±0.22	0.91±0.21	0.7
FBG (mg/dl)	107.25±62.18	121.04±53.18	155.82±96.11	0.1

The data were expressed as Mean ± SD; One-way ANOVA was used to significantly compare between means; Significant difference ($P \leq 0.05$), Highly Significant difference ($P \leq 0.01$), no significant difference ($P > 0.05$); Means followed by different letters are significantly different according to Duncan's multiple range comparisons (DMRTs), Means followed by the same letter are not significantly different; SVD: single vessel disease; 2VD: two-vessels disease; 3VD: three-vessels disease.

Receiver Operating Characteristic (ROC):

The current study detected that *miR133a-5p* showed AUC=0.8 and the best cut off value of 0.94 with 98% sensitivity and 47% specificity, so it is considered a very good biomarker in the diagnosis of those patients as illustrated in Table 4 and Figure. 3. On the other hand, ROC showed that MR-proADM is an excellent diagnostic biomarker for stable angina patients with AUC=1.00, the best cut off value of 213.37, 100% sensitivity and 100% specificity as in Table 4 and Figure.4.

Table 4. Receiver Operating Characteristic curve data of the studied new biomarkers:

Biomarker	AUC	Explanation	*P*-value	The best cut off	Sensitivity %	Specificity %
MR-proADM	1.00	Excellent	0.001	213.37	100	100
miR133a-5p	0.8	Very good	0.001	0.94	98	47

AUC: area under curve.

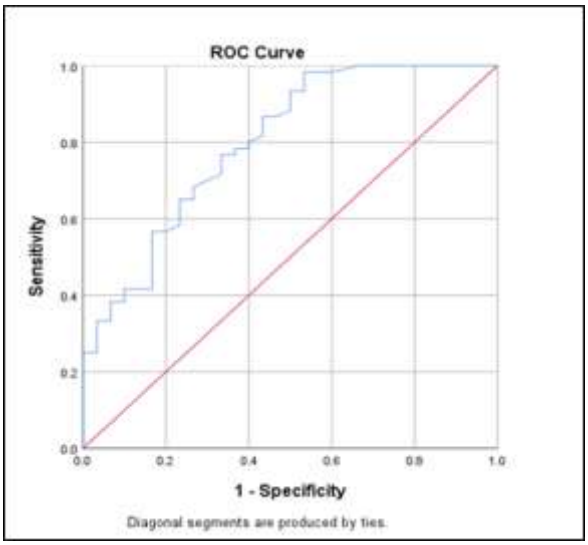

Figure 3. Receiver Operating Characteristic curve of miR133a-5p.

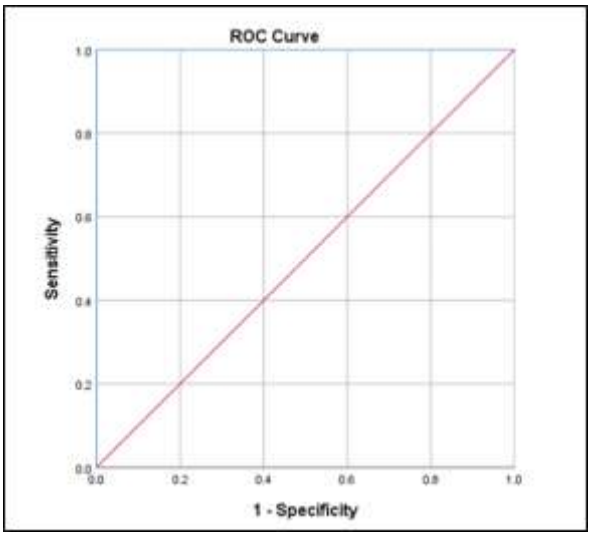

Figure 4. Receiver Operating Characteristic curve of MR-proADM.

Since chronic angina chest discomfort can be similar to that related with other causes of chest pain like lung infections or heart burn related to gastroesophageal reflux disease (GERD), and it is also related to poor prognosis like ischemic heart failure and myocardial infarction, so it needs a rapid

and accurate diagnosis which should be at the same time non-invasive [33,34]. MicroRNAs represent one of the most powerful and precise biomarkers to be effective in the prognosis and diagnosis of many diseases including cardiovascular diseases like CAD and ischemic heart disease [35]. In the current study, the role microRNA 133a-5p in the diagnosis of chronic stable angina patients with obstructive CAD and normal LV ejection fraction had been determined. It was found that the fold of microRNA 133a-5p gene expression was significantly higher in patients than in the control group and ROC curve analysis showed that it had 98% sensitivity and 47% specificity, so it can be used as a diagnostic biomarker that gives a rapid diagnosis for stable angina but with low specificity, therefore it might be of benefit in multi-markers approach.

A study found that it could be used for the prediction of acute MI and arterial hypertension and PCI complications [20,36]. In addition, a study demonstrated that the levels of microRNA 133a were down-regulated in CAD patients [37]. On the other hand, the current study detected that MR-proADM was an excellent diagnostic biomarker for stable angina patients with normal LV ejection fraction with 100% sensitivity and 100% specificity based on ROC analysis, besides, its serum levels were significantly higher in those patients. There were some studies which demonstrated that MR-proADM could be used as a prognostic biomarker for acute MI outcomes and adverse remodeling with LV dysfunction [38,39]. So, most of the previous studies focused on the roles of microRNA 133a and MR-proADM in the prognosis of such patients rather than on its roles in their diagnosis. Regarding the risk factors for having CAD and stable angina, the current study demonstrated no significant effect of age, BMI, smoking status and family history on the susceptibility for having CAD and angina. This result did not agree with the common fact that aging is considered an essential risk factor for heart diseases based on the facts that aging especially when combined with environmental and genetic factors will lead to loss of vessels elasticity, increased stiffness of the arteries, loss of endothelium integrity, increased cyclooxygenase COX1 and COX2 activity, von Willebrand factor and other changes which might limit myocardial perfusion and increase the risk for ischemic heart disease [40–42]. However, there was another study which agreed with this result regarding BMI [43]. Hypertension and dyslipidemia were considered important risk factors, this was agreed with most studies [44–47]. On the other hand, it was detected that the higher percentage of patients did not have DM, this did not agree with a study which stated that DM is considered a major risk factor for having CAD and associated with poor CAD outcomes [48].

It was found that the majority of patients were males which means that males were more prone to have angina than females, this was agreed with most studies [48,49]. The present study detected that microRNA 133a-5p serum levels were affected by the number of diseased coronary vessels, it was higher in patients with single-vessel disease. There were no other studies found to detect the levels of the studied biomarkers and influence by the number of the diseased coronary arteries. However, Gensini score is usually used to assess the occlusion degree in the coronary vessels [50] and Framingham risk score (FRS) is used to predict the severity of CAD [51].

Conclusion

It could be concluded that MR-proADM could be used as an independent biomarker for the diagnosis of stable angina patients with normal LV ejection fraction, whereas microRNA 133a-5p is better to be used in combination with other biomarkers to give more accurate diagnosis.

Acknowledgment

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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Ethics Statements

This study was performed based on the local ethical committee recommendations of the College of Pharmacy/Mustansiriyah University.

Author Contribution

Aseel Ghassan Daoud: samples collection, laboratory analysis, writing and statistical analysis; Ahmed Yousif Hasan: diagnosis with ECG, Echocardiography and coronary angiography; Wassan Abdul Kareem Abbas: supervision, editing and reviewing..

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تحديد مستوى الحمض النووي الريبوسي الصغير١٣٣a-5p والمؤيد الاقليمي للادرينوميدولين في المرضى الذين يعانون من الذبحة الصدرية المستقرة

اسيل غسان داود*[١] ، احمد يوسف حسن[٢] و وسن عبد الكريم عباس[١]

[١] فرع العلوم المختبرية السريرية، كلية الصيدلة، الجامعة المستنصرية، بغداد، العراق

[٢]وزارة الصحة ،المركز العراقي لأمراض القلب، مستشفى الشهيد غازي الحريري، بغداد، العراق

الخلاصة

الذبحة الصدرية المستقرة المزمنة (CSAP) هي مرض قلبي منتشر يمكن تعريفه على أنه عرض أو متلازمة سريرية لمرض الشريان التاجي المستقر (CAD). عادة ما يحدث مرض الشريان التاجي بسبب وجود لويحات تصلب الشرايين أو جلطة دموية داخل الشرايين التاجية التي قد تؤدي إلى نقص التروية. يتم التعبير عن miRNA 133a بشكل رئيسي في العضلات وله دور في موت الخلايا المبرمج وتولد الأوعية والتضخم وتصلب الشرايين ونقص التروية. المؤيد الإقليمي الأوسط للأدرينوميدولين (MR-proADM) هو عضو في عائلة ببتيد الكالسيتونين وسلائف الأدرينوميدولين (ADM) ولكن مع نصف عمر بلازما أطول وأكثر استقرارًا من الناحية الفسيولوجية من الأدرينوميدولين (ADM)، من أصل بطاني ويتم إنتاجه بواسطة مواقع مختلفة في الجسم بشكل رئيسي عن طريق القلب والكلى والرئتين ونخاع الغدة الكظرية. يُعتقد أن مستوى البلازما MR-proADM ينبئ بمرض CAD. كان الهدف من الدراسة الحالية هو الكشف عن مستويات مصل microRNA 133a-5p و MR-proADM في عينة من المرضى العراقيين الذين يعانون من الذبحة الصدرية المستقرة المزمنة و CAD الانسدادي (حيث أن هناك العديد من الأسباب للإصابة بـ CSAP وأحدها هو CAD) مع الكسر القذفي للبطين الأيسر الطبيعي (LVEF) وذلك للتحقق مما إذا كان يمكن استخدام هذه المؤشرات الحيوية لتشخيص هؤلاء المرضى. في دراسة الحالات والشواهد هذه، عينات الدم الوريدية الصيامية (هذا هو البروتوكول، يجب أن يكون المريض صائمًا لأنه أثناء القسطرة، قد يصاب المريض ببعض المضاعفات مثل ثقب أو تشريح أو عدم استقرار الشريان الجذعي الأيسر (LMS)، لذلك المريض يجب إحالته لإجراء عملية جراحية عاجلة) تم جمعها من ٩٠ ذكرًا وأنثى (التعبير المصلي المرجعي لـ microRNA 133a منخفض في كلا الجنسين ومستوى المصل المرجعي لـ MR-proADM عادة ما يكون متشابهًا في كلا الجنسين) (٤٠-٧٦ عامًا)) الذين تم عرضهم على العيادة الخارجية وهم يعانون من آلام في الصدر (تم جمع عينات الدم من المرضى بعد إحالتهم إلى المركز العراقي لأمراض القلب وليس في وقت زيارتهم للعيادة، لذلك تم توجيههم بالحضور صائمين في العيادة يوم تصوير الأوعية في المركز). تم تطبيق تخطيط كهربية القلب وتخطيط صدى القلب وتصوير الأوعية التاجية التقليدية من قبل طبيب القلب لجميع المشاركين. كما تم تطبيق الاختبارات البيوكيميائية الروتينية واستبيان المرضى. تم الكشف عن الكسر القذفي للبطين الأيسر باستخدام طريقة الوضع M ذات السطحين. وفقًا لذلك، تم تصنيف المرضى الذين أظهروا دليلاً على وجود لوحة تصلب الشرايين وCAD اثناء تصوير الأوعية على أنهم مرضى الذبحة الصدرية (العدد = ٦٠) وأولئك الذين لم يظهروا أي دليل على انسداد الشريان التاجي ونقص التروية في تصوير الأوعية (تصوير الأوعية الطبيعي) تم تصنيفهم على أنهم مجموعة مراقبة (العدد = ٣٠). تم قياس أضعاف التعبير الجيني لـ miRNA 133a-5p في المصل و مستويات المصل لـ MR-proADM، وجلوكوز الدم الصائم (FBG)، واليوريا، والكرياتينين، وTGs، والكوليسترول الكلي، وLDL-C، وHDL-C، وVLDL-C. النتائج: كانت طية التعبير الجيني miRNA 133a-5p في المصل ومستويات المصل لـ MR-proADM أعلى بكثير في المرضى ($P \geq 0.01$) وأظهر منحنى ROC أن miRNA 133a-5p كان لديه حساسية بنسبة ٩٨٪ وخصوصية ٤٧٪ مع AUC. =0.8، في حين أن MR-proADM كان لديه حساسية ١٠٠٪ وخصوصية ١٠٠٪ مع AUC=1.0. لذلك، يمكن أن نستنتج أنه يمكن استخدام MR-proADM كمؤشر حيوي مستقل لتشخيص مرضى الذبحة الصدرية المستقرة الذين لديهم جزء قذفي طبيعي من البطين الأيسر، في حين من الأفضل استخدام microRNA 133a-5p مع المؤشرات الحيوية الأخرى لإعطاء تشخيص أكثر دقة.

الكلمات المفتاحية: الذبحة, تصلب الشرايين, تشخيص, الحمض النووي الرايبوسي الصغير١٣٣a-5p, المؤيد الاقليمي للادرينوميدولين