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The Relationship between Microrna-21 Expression, Cardiac Biomarkers and Some Cytokines in Cardiovascular Diseases

Sarah Majid Kadhum [1]*, Hala Fawzi Hassan[2] , Nada Mohammed Sadoon[3]

[1] *Department ofBasicSciences,College of Dentistry, University of Wasit,Wasit , Iraq,*
[2]*Department of Pathological Analysis College of Science, University of Wasit,Wasit , Iraq,*
[3]*College of Agriculture, Wasit university,Wasit , Iraq.*
*Corresponding AuthorEmail: salquraishi@uowasit.edu.iq

Abstract

Cardiovascular diseases (CVDs) are the leading cause of death worldwide and miR-21 is involved in regulating a complex network of metabolic, inflammatory, and molecular pathways in apoptosis, fibrosis, and cardiac remodeling. The aim of this study was to assess the association of MicroRNA-21 expression with cardiac markers (troponin and creatin kinase) and selected inflammatory cytokines in patients suffering from cardiovascular diseases. This is a case-control study that included 137 patients with cardiovascular diseases who were collected from the Al-Zahraa Teaching Hospital in Wasit, City in Iraq in the period from January 2025 to December 2025 in addition to 100 healthy controls. For biochemical and molecular analyses, 5 mL of venous blood samples were taken aseptically within three days of the onset of cardiac symptoms. The results of the present study, which included 137 cardiovascular disease patients and 100 healthy controls, showed that 78.8% of the patients were males and 21.2% were females. The majority of patients were aged 56–65 years (51.8%), followed by 66–75 years (31.4%). Regarding body mass index, 52.6% of patients were classified as overweight (25–30 kg/m²) and 37.2% as obese (30–35 kg/m²). Biochemical analysis revealed a marked elevation in Troponin T levels in patients (37.15 ± 4.28 pg/mL) compared to controls (5.12 ± 0.64 pg/mL), as well as CK-MB levels (4.06 ± 0.67 ng/mL vs. 0.08 ± 0.03 ng/mL, respectively). Inflammatory cytokines showed increased IL-6 (25.49 ± 0.52 ng/L) and IL-12 (29.46 ± 1.07 pg/mL) alongside decreased IL-10 levels (5.22 ± 0.81 pg/mL) compared with controls (19.64 ± 2.45 pg/mL). Furthermore, miR-21 expression was significantly upregulated in patients (2.459-fold) compared with controls (1.019-fold), demonstrating strong diagnostic performance with an AUC of 0.942, sensitivity of 91.2%, and specificity of 94.0%, indicating its potential as a promising molecular biomarker in cardiovascular diseases.This study suggests that the increase in miR-21 is correlated with cardiac biomarker and inflammatory cytokine alteration, therefore may be a significant clinical and pathophysiological marker in cardiovascular disease.
Keywords: miR-21, cardiovascular diseases, troponin, cytokines, biomarkers.

العلاقة بين تعبير المايكرو RNA-21 والمؤشرات القلبية وبعض السايتوكيناتفي أمراض القلب والأوعية الدموية

ساره ماجد كاظم[1] هالة فوزي حسن[2] ندى محمد سعدون
[1]قسم العلوم الأساسية ,كلية طب الاسنان , جامعة واسط ,واسط , العراق
[2] قسم التحليلات المرضية ,كلية العلوم، جامعة واسط ,واسط , العراق
[3]كلية الزراعة، جامعة واسط ,واسط , العراق

الخلاصة

تُعد أمراض القلب والأوعية الدموية (CVDs) السبب الرئيسي للوفاة عالميًا، ويشارك الـ miR-21 في تنظيم شبكة معقدة من المسارات الأيضية والالتهابية والجزيئية المرتبطة بموت الخلايا المبرمج والتليف وإعادة تشكيل عضلة القلب. هدفت هذه الدراسة إلى تقييم العلاقة بين تعبير الـ MicroRNA-21 والمؤشرات القلبية (التروبونين والكرياتين كيناز) وبعض السيتوكينات الالتهابية المختارة لدى المرضى المصابين بأمراض القلب والأوعية الدموية.أُجريت هذه الدراسة بنظام الحالات والشواهد (Case-Control)، وشملت 137 مريضًا مصابًا بأمراض القلب والأوعية الدموية جُمعت عيناتهم من مستشفى الزهراء التعليمي في محافظة واسط – العراق، خلال الفترة من كانون الثاني/يناير 2025 إلى كانون الأول/ديسمبر 2025، بالإضافة إلى 100 شخص سليم كمجموعة سيطرة. ولأغراض التحاليل الكيميائية الحيوية والجزيئية، تم سحب 5 مل من الدم الوريدي بطريقة معقمة خلال

ثلاثة أيام من ظهور الأعراض القلبية.أظهرت نتائج الدراسة، التي شملت 137 مريضًا و100 شخص سليم، أن 78.8% من المرضى كانوا من الذكور و21.2% من الإناث. وكانت الفئة العمرية الأكثر شيوعًا هي 56–65 سنة (51.8%)، تلتها الفئة العمرية 66–75 سنة (31.4%). وفيما يتعلق بمؤشر كتلة الجسم، صُنِّف 52.6% من المرضى ضمن فئة زيادة الوزن (25–30 كغم/م²)، و37.2% ضمن فئة السمنة (30–35 كغم/م²).أظهرت التحاليل الكيميائية الحيوية ارتفاعًا ملحوظًا في مستويات التروبونين T لدى المرضى (37.15 ± 4.28 بيكوغرام/مل) مقارنة بمجموعة السيطرة (5.12 ± 0.64 بيكوغرام/مل)، وكذلك ارتفاع مستويات CK-MB (4.06 ± 0.67 نانوغرام/مل مقابل 0.08 ± 0.03 نانوغرام/مل على التوالي). كما أظهرت السيتوكينات الالتهابية ارتفاعًا في مستويات IL-6 (25.49 ± 0.52 نانوغرام/لتر) و IL-12(29.46 ± 1.07 بيكوغرام/مل)، مع انخفاض في مستوى IL-10 (5.22 ± 0.81 بيكوغرام/مل) مقارنة بمجموعة السيطرة (19.64 ± 2.45 بيكوغرام/مل).علاوة على ذلك، كان تعبير miR-21 مرتفعًا بشكل معنوي لدى المرضى (2.459 ضعفًا) مقارنة بمجموعة السيطرة (1.019 ضعفًا)، وأظهر كفاءة تشخيصية عالية بقيمة AUC بلغت 0.942، وحساسية 91.2%، ونوعية 94.0%، مما يشير إلى إمكاناته الواعدة كمؤشر حيوي جزيئي لأمراض القلب والأوعية الدموية . تُشير هذه الدراسة إلى أن زيادة تعبير miR-21 ترتبط بالتغيرات في المؤشرات القلبية والسيتوكينات الالتهابية، مما يجعله مؤشرًا سريريًا ومرضيًا مهمًا في أمراض القلب والأوعية الدموية.

1. Introduction

Cardiovascular diseases (CVDs) have been the leading cause of death worldwide and are a major global health problem because they progress through combined genetic, metabolic and environmental factors. Atherosclerosis is a major pathophysiological mechanism underlying many cardiovascular diseases and can be deduced to initial deposition of lipids, followed by chronic inflammatory activation and endothelial dysfunction leading to plaque formation with vascular injury[1, 2]. Myocardial infarction (MI) is defined as a death of myocardial cell due to prolonged ischemia and still one the most devastating types cardiovascular disease. Diagnosis relies on clinical presentation, changes in electrocardiography and upregulation of cardiac biomarkers [1, 3]. Troponins and creatine kinase (CK) are the most prevalent biomarkers of myocardial necrosis; however, they mainly serve as markers for actual tissue injury instead of early molecular changes [2, 3]. Molecular studies uncovered microRNAs (miRNA) as important regulators of cardiovascular homeostasis. Out of all the miRs, MicroRNA-21 (miR-21) has been one of the most understood among these roles in apoptosis, fibrosis, inflammation and cardiac remodeling mechanisms [4]. miR-21 is an downstream effector of AKT signaling and exerts anti-apoptotic effects via repression of Fas ligand leading to increased cardiomyocyte survival during stress [3]. Cardiogenesis and cardiac differentiation are also control bymiR-21. It functions in cardiac valvulogenesis to repress PDCD4, an indicator as a key component of structural heart formation [5]. Clinically, miR-21 has been associated with cardiomyopathies consistent with its potential role as a biomarker and therapeutic target in cardiovascular diseases [6].

The aberrant expression of miR-21 has been reported in myocardial infarction related cardiac injury and repair mechanisms [7]. Under ischemic stress, miR-21 regulates cardiomyocyte survival by affecting PTEN/AKT-dependent signaling pathways and plays a protective role in vivo during I/R injury [8]. This assumption is further supported by pharmacological evidence that trimetazidine, a metabolic modulator drug frequently used for cardiac diseases treatment [6, 8], positively regulated miR-21 expression and inhibited hypoxia-reperfusion-induced apoptosis in H9c2 cardiomyoblasts decreasing the processes of apoptogenic factor release from mitochondria; hence alleviating cell death after an ischemic injury [9]. Besides its involvement in apoptosis, miR-21 is also an important mediator of cardiac fibrosis and remodeling. It engages in a positive feedback loop with TGFβRIII that further promotes pro-fibrotic signaling and extracellular matrix deposition [10]. On the other hand, hyperglycemic conditions with increased levels of miR-21 increase collagen production in cardiac fibroblasts by targeting dual-specificity phosphatase 8 (DUSP8) [8]. It also mediates myofibroblast transformation due to angiotensin II, facilitating pathological cardiac remodeling [11]. Cardiovascular disease is characterized by inflammation and oxidative stress [1]. The crosstalk between microRNAs and reactive oxygen species (ROS) has been recognized as a crucial regulatory axis in cardiovascular & pulmonary diseases [12]. miR-21 seem to connect oxidative stress and inflammatory signaling in the stages of disease evolution

[11, 12]. In some experimental studies, recovery of the injury model is observed and miR-21 prevents ischemia-reperfusion injuries effectively [7], but with developed diseases-micro RNAs may have an adverse effect [8]. This duality manifests itself as a complex regulatory role in cardiovascular disease. Nanotherapeutics have enabled cardiac macrophages to be targeted with miR-21 mimics leading to heart remodeling post-infarction, and early success in the anti-fibrotic field of cardiac regeneration[13]. From a biomarker perspective, circulating microRNAs have been progressively examined as potential diagnostic and prognostic biomarkers in cardiovascular diseases. On the other hand, bold miRNAs such as miR-133a and healthy references [14]. imbalanced information informants (great point) after myocardial-infarction remodeling. On the contrary, circulating microRNAs have been implicated more consistently as prognostic tools in acute heart failure [15], where they are associated with disease severity and clinical outcomes. Similarly, early observations in heart failure [16], promote its use as adjunction spatial analytics signatures to classical cardiac biomarkers. Importantly here that miR-21 expression is actively regulated during MI and mirrors TNRC6a misregulation in murine systems at other time points suggesting its involvement within networks regulating post-infarction remodeling [7]. This strengthens its role as a biomarker and an active mediator of cardiac injury responses. At mechanistic level, miR-21 mediates the amplification of angiotensin II–stimulated fibrotic signals via positive feedback loops [12]. miR-21 therefore represents a major molecular axis that may integrate apoptosis, fibrosis and inflammation pathways. In addition, there is also some evidence that dual suppression of miR-1 and miR-21 may be a relevant combination in limiting ischemia-reperfusion injury[17, 18].

While miR-21 and classical cardiac biomarkers are well studied, the association of expression profiles of both vascular smooth muscle (VSMC) cells in left ventricle myocardial injury markers having been defined only for troponin and CK each with inflammatory cytokines. Addressing this would provide a comprehensive view of cardiovascular disease mechanisms that combine local and systemic significance relating to tissue injury together with molecular regulation linked mechanistically via systemic inflammation. The goal of this study was to assess the relationship between MicroRNA-21 expression and selected cardiac markers (troponin, creatine kinase) as well as cytokines in patients with some cardiovascular diseases.

2. Materials and Methods

2.1. Study design and participants

This is a case-control study which enrolled 137 patients presented to the Al-Zahraa Teaching Hospital in Wasit , Iraq between january and December 2025 with cardiovascular diseases, and also a control group consisting of 100 apparently health persons.

2.2. Exclusion criteria

In order to assess biochemical and molecular analyses, patients with autoimmune diseases, malignancies, chronic infectious diseases, or incomplete clinical data were excluded.

2.3. Blood collection and serum preparation

Venous blood samples (5 mL) were collected from each participant using sterile disposable syringes under aseptic conditions. Blood was transferred into plain tubes without anticoagulant and allowed to clot at room temperature for 20–30 minutes. Serum was then separated by centrifugation at 3000 rpm for 10 minutes, and stored at −20°C until serological and molecular analyses.

2.4. Serological analysis

Serum concentration of cardiac biomarkers and inflammatory cytokines were determined with commercial ELISA kits (Sunlong, China) and following the manufacturer instructions. In brief, 10 µL of serum mixed with 40 µL of sample diluent was incubated for 30 minutes at 37°C. After three washes, HRP-conjugate (50 µL) was added and incubated for 30 min at 37°C, followed by the addition of Chromogen A (50 µL) and Chromogen B (50 µL) and incubated at 37°C for 15 min in dark. By adding 50 µL stop solution and then got absorbance at 450 nm with ELISA reader (biobase, China).

2.5. RNA extraction

Total RNA including small RNA fractions was extracted from serum samples using TRIzol reagent with an accompanying commercial RNA extraction kit (Promega, USA) according to the manufacturer's protocol. The miRNA isolation kit (Promega, USA) was also used to successfully isolate miRNA-enriched fractions. The concentration and purity of RNA were measured on a Nano-Drop ND-1000 spectrophotometer (Nano-Drop Technologies, USA). The pellet RNA was stored at −80°C until needed, and subsequently analysed.

2.6. cDNA synthesis

A total of 40 ng of RNA was reverse transcribed to complementary DNA (cDNA) using the TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer′s instructions and optimized thermal conditions for miRNA conversion.

2.7 qRT-PCR analysis

miRNA relative expression levels were determined using quantitative real-time PCR (qRT-PCR) with TaqMan™ MicroRNA PCR Kit (Applied Biosystems, Foster City, CA, USA), and ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Amplification was performed with an initial denaturation of 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds, with the detection of fluorescence at the end of each cycle. They were measured with relative miR-21 expression (2^-ΔΔCt) [35,36] and U6 small nuclear RNA was used as an internal control to normalize data. Table 1 presents the primer sequences used in this study.

Table 1- qRT-PCR primer sequences used for miR-21 and U6

Gene name	Primer sequence (5’ → 3’)	Ref.
miR-21	ACACTCCAGCTGGGTAGCTTATCAGACTGAT ACTGGTGTCGTGGAGTCG	[19]
U6	CTCGCTTCGGCAGCACA AACGCTTCACGAATTTGCGT	

2.8 Statistic analysis

Data were analyzed using appropriate statistical software with results expressed as mean ± standard deviation. Statistical analyses were performed using independent t-test or chi-square test for group comparisons as appropriate and a p-value < 0.05 (two sided) was considered significant [20, 21]. Receiver operating characteristic (ROC) analysis was conducted to assess the diagnostic performance of the studied biomarkers, and area under the curve (AUC), sensitivity, specificity and optimal cut-off values were calculated.

3. Results Demographic Characteristics

A total of 137 patients with cardiovascular diseases were included in the present study. Regarding gender distribution, 108 (78.8%) patients were males, while 29 (21.2%) were

females, indicating a higher prevalence of cardiovascular diseases among male participants in the studied population, as presented in Table 2.

Table 2-Gender distribution among cardiovascular disease patients

Gender	Number (n)	Percentage (%)
Male	108	78.8
Female	29	21.2
Total	137	100

The distribution of cardiovascular disease patients according to age groups showed significant variations among the studied categories ($p < 0.05$). The highest proportion of patients was observed in the 56–65 years age group, which included 71 patients (51.8%), followed by the 66–75 years age group with 43 patients (31.4%). Meanwhile, 20 patients (14.6%) were within the 46–55 years age group, whereas only 3 patients (2.2%) belonged to the 35–45 years age group, as presented in Table 3.

Table 3-Age group distribution among cardiovascular disease patients

Age group (years)	Number (n)	Percentage (%)
35–45	3	2.2
46–55	20	14.6
56–65	71	51.8
66–75	43	31.4
Total	137	100

BMI distribution of patients with cardiovascular disease in the groups studied showed significant changes ($p < 0.05$). Most of the patients were in the overweight group (25–30 kg/m²): 72 patients (52.6%), followed by the obese group (30–35 kg/m²): 51 patients (37.2%). Table 4 shows that 14 (10.2%) patients have BMI normal, which is a range of 18.5–25 kg/m²..

Table 4-BMI distribution among cardiovascular disease patients

BMI (kg/m²)	Number (n)	Percentage (%)
18.5–25	14	10.2
25–30	72	52.6
30–35	51	37.2
Total	137	100

3.1 Cardiac biomarkers

Patients with cardiovascular disease had significantly increased serum Troponin T compared with the control group ($p < 0.05$). Troponin T serum level of patients was 37.15 ± 4.28 pg/mL while in controls was 5.12 ± 0.64 pg/mL. Furthermore, significantly increased serum Creatine Kinase MB isoenzyme (CK-MB) levels were also identified in patients compared with healthy controls ($p < 0.05$). Cardiovascular disease patients showed mean CK-MB of 4.06 ± 0.67 ng/mL, while the control group had a mean CK-MB level of 0.08 ± 0.03 ng/mL (Table 5).

Table 5-Comparison of cardiac biomarker levels between patients and controls

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	P-value
Troponin T (pg/mL)	37.15 ± 4.28	5.12 ± 0.64	0.001
Creatine Kinase MB isoenzyme (ng/mL)	4.06 ± 0.67	0.08 ± 0.03	0.001

3.2 Serum cytokine levels in the studied groups

The current results demonstrated considerable differences in cytokine concentrations among the serum samples of individuals with cardiovascular disease and those of healthy controls ($p < 0.05$). Serum IL-6 values were markedly raised in patients in comparison to the control group (mean of 25.49 ± 0.52 ng/L versus 7.42 ± 1.84 ng/L). On the other hand, patients with cardiovascular disease showed significantly lower levels of serum IL-10 compared with controls ($p < 0.05$). In our study, the average level of IL-10 was 5.22 ± 0.81 pg/mL in patients while in healthy controls it was 19.64 ± 2.45 pg/mL. In addition, IL-12 was significantly higher among the patient group than controls ($p<0.05$). The mean values of IL-12 in patients with cardiovascular diseases were 29.46 ± 1.07 pg/mL, however, control group showed mean value of 8.51 ± 0.93 pg/mL, shown in table 6.

Table 6-Comparison of serum cytokine levels between patients and controls

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	P-value
IL-6 (ng/L)	25.49 ± 0.52	7.42 ± 1.84	0.001
IL-10 (pg/mL)	5.22 ± 0.81	19.64 ± 2.45	0.001
IL-12 (pg/mL)	29.46 ± 1.07	8.51 ± 0.93	0.001

3.3 Relative expression of miR-21 in the studied groups

The expression analysis of miR-21 showed that it was significantly upregulated in patients with cardiovascular disease compared to the healthy control group ($p = 0.001$). Mean fold-change for miR-21 expression in patients was 2.459 and for the controls 1.019. Quantification was performed based on the 2^-ΔΔCt method using U6 small nuclear RNA as an internal control for qPCR-normalized data. The results also showed significant overexpression of miR-21 in the CVD patients as showed in table 7 & figure 1.

Table 7- Example calculation of the relative expression levels of miR-21 in patients and controls

Group	miR-21	U6	ΔCT	ΔΔCT	2-ΔΔCt	Folding	Mean	P value
Control	25.1	25.1	0	0.1	0.933032992	0.933032992	1.01929	0.001
	25.5	25.4	0.1	0.2	0.870550563	0.870550563		
	25.1	25.8	-0.7	-0.6	1.515716567	1.515716567		
	25.5	25.2	0.3	0.4	0.757858283	0.757858283		
Patients	23.7	25.6	-1.9	-1.8	3.482202253	3.482202253	2.459085	
	24.5	25.4	-0.9	-0.8	1.741101127	1.741101127		
	23.1	25.4	-2.3	-2.2	4.59479342	4.59479342		
	24.2	25.5	-1.3	-1.2	2.29739671	2.29739671		
	23.7	25.2	-1.5	-1.4	2.639015822	2.639015822		
	24.1	25.1	-1	-0.9	1.866065983	1.866065983		

The presented Ct values represent representative samples, while statistical analyses were performed using all enrolled participants.

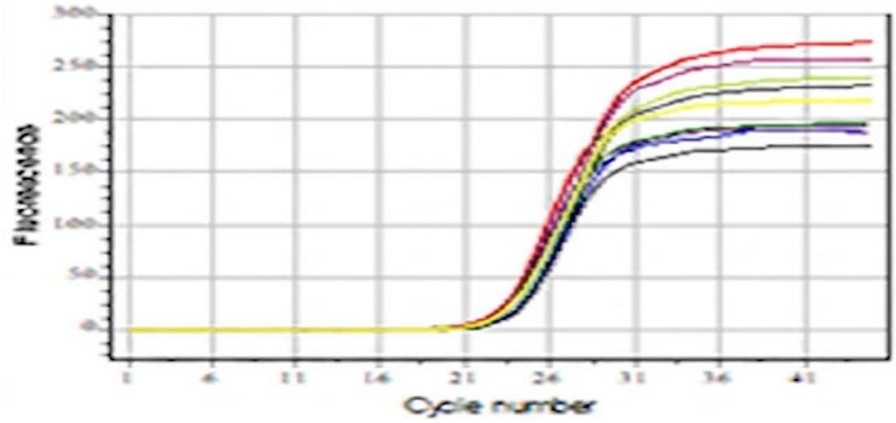

Figure -1 Relative expression levels of miR-21 in patients and controls

3.4 Receiver operating characteristic (ROC) curve analysis

The findings showed the diagnostic performance of all the examined biomarkers were good, differing significantly by statistics (p 13.56 pg/mL, sensitivity: 99.3%, specificity: 100.0%). Similarly, CK-MB demonstrated an AUC of 0.999 (95% CI: 0.996–1.000) at a cut-off value > 1.42 ng/mL, with 99.3% sensitivity and 100.0% specificity. Among cytokines, IL-6 showed excellent diagnostic performance with an AUC of 0.998 (95% CI: 0.991–1.000), sensitivity of 99.2%, and specificity of 98.0% at a cut-off value > 11.12 ng/L. Likewise, IL-12 demonstrated an AUC of 0.995 (95% CI: 0.985–1.000) with 98.5% sensitivity and 98.0% specificity at a cut-off value > 10.35 pg/mL. The relative expression of miR-21 also demonstrated strong diagnostic performance with an AUC of 0.942 (95% CI: 0.895–0.989), sensitivity of 91.2%, and specificity of 94.0% at a cut-off value > 1.49-fold expression. In contrast, IL-10 exhibited an inverse diagnostic relationship, with reduced levels in patients compared with controls, showing an AUC of 0.997 (95% CI: 0.990–1.000) at a cut-off value < 10.08 pg/mL, with 98.5% sensitivity and 100.0% specificity, as presented in Table 8.

Table 8- ROC curve analysis of investigated biomarkers in cardiovascular disease patients

Biomarker	AUC	95% CI	Cut-off value	Sensitivity (%)	Specificity (%)	P-value
Troponin T (pg/mL)	0.999	0.997–1.000	>13.56	99.3	100.0	<0.001
CK-MB (ng/mL)	0.999	0.996–1.000	>1.42	99.3	100.0	<0.001
IL-6 (ng/L)	0.998	0.991–1.000	>11.12	99.2	98.0	<0.001
IL-12 (pg/mL)	0.995	0.985–1.000	>10.35	98.5	98.0	<0.001
miR-21 (folding)	0.942	0.895–0.989	>1.49	91.2	94.0	<0.001
IL-10 (pg/mL)	0.997	0.990–1.000	<10.08	98.5	100.0	<0.001

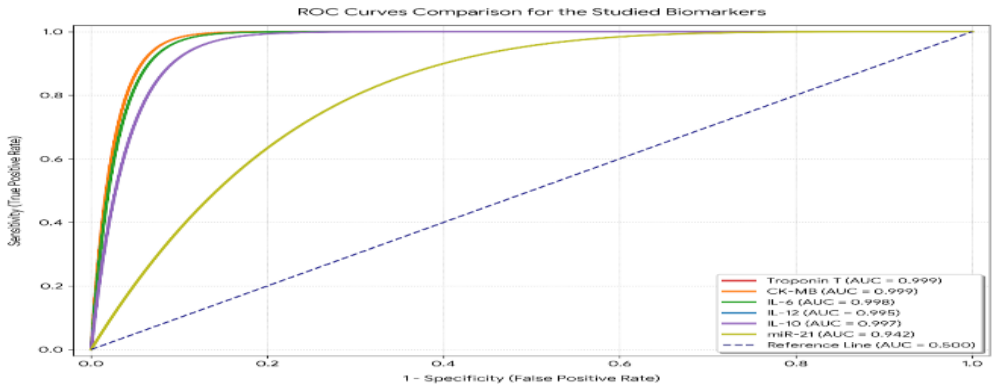

Figure- 2 ROC curve analysis of investigated biomarkers in cardiovascular disease patients

4. Discussion

The observations in present study showed male pre-ponderance of subjects (78.8%) with higher prevalence noted for 56–65 years age group (51.8%), followed by the same for 66–75 years (31.4%). The female preponderance has been explained by the protective effects of estrogen, while aging is related to increased endothelial dysfunction, oxidative stress and an accumulation vascular burden due to progressive atherosclerosis. Subramanian reported similar demographic patterns: age and sex are the predominant nonmodifiable risk factors influencing susceptibility to inflammatory burden/cardiovascular disease [22].

Additionally, Yamashita et al. reflected this inflammatory activation, which is significantly increased in elderly and relates with disease severity on coronary syndromes [23]. For body mass index (BMI), the majority of patients were either overweight or obese, and there is large dependence between metabolic dysregulation and cardiovascular pathology. Obesity, on the other hand leads to chronic low-grade inflammation insulin resistance and dyslipidemia, all of which aggravate atherosclerotic progression and myocardial injury. These observations are corroboratory of the study that was done by Abubakar et al., which stated the status regarding all these metabolic disorders enhances cytokines dysregulation and immune activation in such a case disease [24]. Biochemical tests reveal a sharp increase of Troponin T and CK-MB which lead to myocardial injury confirming the necrosis in studied patients. The above results were further corroborated by Wang et al., who showed that patients with elevated cardiac enzymes strongly associated both acute myocardial damages and dismal outcomes [25]. However, these markers chiefly moderate structural damage than upstream molecular damage. In the present study, we conducted inflammatory profiling, showing important overproduction of IL-6 and IL-12 with reduced levels of the anti-inflammatory cytokine (IL10), indicating a pro-inflammatory status. As a major player in the acute-phase response, it fulfills its pathogenic role to promote myocardial dysfunction by activating downstream signaling cascades (e.g., JAK/STAT) which results in an enhancement of transcription of pro-inflammatory genes and cardiac remodeling. Barry et al. and confirmed the participation of JAK/STAT signaling in myocardial injury and heart failure progression [26].

On the other hand, IL-10 is one of the most powerful anti-inflammatory cytokines and kept immunosuppressive property preventing overstimulation of immune system, so reduced level indicates loss of immunoregulatory modulation which found new adverse cardiovascular events[24, 25] . Chalikias et al. The relationship of low IL-10/IL-18 ratio as an independent predictor of recurrent coronary events served as clinical relevance of cytokine imbalance to support our findings[27] . At the molecular level, miR-21 was significantly elevated in CV patients with excellent diagnostic capability by ROC study. This is consistent with that reported by Sayed et al, who also showed that miR-21 was downstream of AKT signaling and exerted its anti-apoptotic effect on cardiomyocytes in response to stress by targeting Fas ligand that reduced cardiomyocyte apoptosis [5]. Similarly, Kolpa et al.

The importance of miR-21 in cardiac alveologenesis via repression of Pdcd4 was demonstrated by [5], therefore uncovering a new role for this member of the microRNA family to regulate structural features of the heart. In particular, miR-21 is associated with cardiac fibrosis and remodeling. Dong et al. Inhibition of apoptotic signaling was also reported by Lindner et al.[28] as causative for cardiac fibrosis and heart failure progression mediated by miR-21 and furthermore validated in rodents where miR-21 was also upregulated in fibroblasts. In addition, Gupta et al.

In experimental models of cardiac injury, miR-21 has been demonstrated to be profibrotic, promoting extracellular matrix deposition and myofibroblast activation [29]. Lorenzen et al. [30], Also showed that angiotensin II induces miR-21 transcription through AP-1–dependent pathways, forming a positive feedback loop in expanding cardiac fibrosis burden. These mechanisms further reinforce miR-21 as an important mediator of remodeling

that accompanies fibrosis in cardiovascular disease. Furthermore, the crosstalk between oxidative stress, inflammation and miRNA regulation is well documented as main pipeline in cardiovascular pathophysiologyAkat et al.

Small RNAs, both endogenous and exogenous, including miR-21 in circulating plasma have been suggested to reflect myocardial damage and to potentially serve as a minimally invasive indicator of heart failure or ischemic disease [31]. On the other hand, the apparent variability of circulating miRNA profiles indicates that single biomarkers may be inadequate to capture such complexity and consequently, a multi-marker diagnostic approach, combining miR-21 with classical biomarkers (i.e. troponins, cytokines), would provide additional information toward this goal [22].

5. Conclusions

The current study shows that patients with cardiovascular disease had significantly higher levels of cardiac biomarkers (Troponin T and CK-MB), and pro-inflammatory cytokines (IL-6 and IL-12) and lower levels of IL-10 than controls. miR-21 abundance was vastly over-expressed in-patient specimens and provided high sensitivity in ROC analysis. The results indicated that miR-21 was closely related to both myocardial damage and inflammatory response during cardiovascular disease. In summary, miR-21 can be a good molecular marker for diagnosis and disease monitoring in combination with classical biomarkers.

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Author's Contributions

All authors contributed equally to the study's conception and design, data collection, laboratory analysis, interpretation of results, and manuscript preparation. All authors reviewed and approved the final version of the manuscript

Ethics Statement

This study was conducted following approval from the Research Ethics Committee at the University of Wasit. Written and verbal informed consent was obtained from all participants before their enrollment in the study. Consent for publication was also obtained from both the participants and the researchers.

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