miRNA miRNA on the Wall, Who Is the Most Cardio-Specific of Them All? Circulating microRNAs as Potential Biomarkers in Acute Coronary Syndrome

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Systematic Review

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Abstract

The severe and acute manifestation of coronary artery disease (CAD) is acute coronary syndrome (ACS); therefore, prompt diagnosis can save lives. Cardiac biomarkers that are accepted to use in evaluating ACS are creatine kinase muscle/brain subtype (CK-MB), cardiac troponin I (CTnI), or cardiac troponin T (CTnT). However, these markers have several drawbacks, such as prolonged time to rise for prompt diagnosis and elevation in patients with chronic kidney diseases (CKD). Lately, potential, novel candidates for cardiac ischemia biomarkers have been developed, one of which is micro-ribonucleic acids (miRNAs). miRNAs are potential due to their remarkable reproducibility and stability. Several miRNAs, such as, miR-1, miR-133a, miR-133b, miR-208a, miR-208b, and miR-499a, greatly rise in concentration in the plasma or serum of patients with acute cardiac ischemia, signifying their cardiac specificity and promising biomarkers in patients with ACS. This systematic review aims to elucidate the role of cardio-specific miRNA in acute myocardial ischemia (AMI) and its relationship with other cardiac biomarkers.

Introduction

Coronary artery disease (CAD), also recognized as ischemic heart disease (IHD), is the most common cardiovascular disease [1]. Acute coronary syndrome (ACS) is elicited by a complete or partial thrombosis of an artery following the rupture of an atherosclerotic plaque and; ACS attributes to an array of clinical presentations encompassing unstable angina, acute myocardial infarction (AMI), and either ST-segment elevation MI (STEMI) or non-ST-segment elevation MI (NSTEMI) [2]. In general, creatine kinase brain/muscle subtype (CK-MB), cardiac troponin I (CTnI), or cardiac troponin T (CTnT) are employed as biomarkers to assess and diagnose AMI prognosis. However, these markers have several drawbacks. CTnI or CTnT starts to increase in three to four hours, followed by CK-MB that increases in four to six hours following the onset of myocardial injury. These biomarkers are also elevated in patients with chronic kidney diseases (CKD) and can be deceiving even when myocardial ischemia is not clinically suspected [3]. Therefore, a clinical demand for a new biomarker, which possesses the ability to rule out or rule in AMI promptly, is increasing. Micro-ribonucleic acids (miRNAs) appear to be a likely candidate for the prompt diagnosis of AMI.

miRNAs are short (~22 nucleotides) endogenous RNAs. miRNAs consisted of about 22 nucleotides, more or less, endogenous RNAs. Thousands of miRNAs have been identified in humans, and they are taught to control one-third of humans' genes. Multiple of them are have been involved in prevalent human pathological conditions [4]. The exceptional stability of miRNAs in urine and blood made them appealing candidates as biomarkers for numerous diseases. Several miRNAs, such as, miR-1, miR-133a/b, miR-208a/b, and miR-499a, were disclosed as greatly elevated in the plasma or serum of patients with AMI [5]. This review aims to elucidate the role of cardio-specific miRNAs in ACS and their relationship with conventional cardiac biomarkers. It also assesses and appraises the capacity of miRNAs as valuable diagnostic biomarkers for prompt diagnosis of AMI.

Materials, Methods, And Results

The search was done by using online libraries: Excerpta Medica database, Medline database, and Cochrane library (EMBASE/PubMed/Cochrane library). Medical subject headings (MeSH) terms and following keywords were applied: “Acute coronary syndrome,” “ACS,” “Acute myocardial infarction,” “AMI,” “microRNA,” “miRNA” and “miR.” Studies published in English, include specimens of humans, carried out from January 2010 onwards, and include patients with acute coronary syndrome (ACS) as an outcome are all included. Studies containing valvular heart disease or congenital heart disease are being excluded. Only case-control studies are included in this article, as many as 20 in number. Additionally, references of all publications from the initial search are also included for supplementary sources.

After a careful investigation, as many as 20 clinical studies were covered in this review. In total, 3560 subjects were included. The source of extraction of microRNA was plasma in 13 studies. The source of microRNA extraction was plasma in six studies, but only one study (Wang et al., 2011) used both plasma and whole blood as the source of microRNA extraction [6].

Table 1. A comparison table of clinical studies which investigated cardiac-specific microRNAs in acute coronary syndrome populations

No.	Author	Year	Study Population	Type	Outcome
1.	Wang et al. [7]	2010	AMI (n=33); Non AMI (n=33)	Case Control	ACS
2.	Adachi et al. [8]	2010	AMI (n=9); UAP (n= 5); CHF III (n=9); CHF II (n=6); Control (n=10)	Case Control	ACS
3.	Corsten et al. [9]	2010	AMI (n=32); Control (n=36)	Case Control	ACS
4.	D'Alessandra et al. [10]	2010	AMI (n=33); Control (n=17)	Case Control	ACS
5.	Ai et al. [11]	2010	AMI (n=93); Control (n=66)	Case Control	ACS
6.	Cheng et al. [12]	2010	AMI (n=31); Control (n=20)	Case Control	ACS
7.	Wang et al. [6]	2011	AMI (n=51); Control (n=28)	Case Control	ACS
8.	Widera et al. [13]	2011	STEMI (n=196); NSTEMI (n=131); Unstable angina (n=117)	Case Control	ACS
9.	Kuwabara et al. [14]	2011	ACS (n=29); Control (n=42)	Case Control	ACS
10.	Gidlof et al. [15]	2011	AMI (n=25); Control (n=11)	Case Control	ACS
11.	Devaux et al. [16]	2012	STEMI (n=397); NSTEMI (n=113); Control (n=87)	Case Control	ACS
12.	Olivieri et al. [17]	2012	NSTEMI (n=92); CHF (n=81); Control (n=99)	Case Control	ACS
13.	Orleman et al. [18]	2012	ACS (n=106); Non ACS (n=226)	Case Control	ACS
14.	Li et al. [19]	2013	AMI (n=117); AP (n=182); Control (n=100)	Case Control	ACS
15.	Gidlof et al. [20]	2013	AMI (n=319); Non AMI (n=88)	Case Control	ACS
16.	Li YQ et al. [21]	2013	AMI (n=67); Control (n=32)	Case Control	ACS
17.	Chen et al. [22]	2014	AMI (n=53); UA (n=20); Control (n=30)	Case Control	ACS
18.	Zhao et al. [23]	2015	AMI (n=59); Control (n=60)	Case Control	ACS
19.	Bialek et al. [24]	2015	STEMI (n=19); Stable CAD (n=12); Control (n=8)	Case Control	ACS
20.	Agiannitopoulos et al.	2018	AMI (n=80); Control (n=50)	Case	ACS

[25]	Control

Note: AMI = acute myocardial infarction; ACS = acute coronary syndrome; UAP = unstable angina pectoris; CHF = chronic heart failure; STEMI = ST elevation myocardial infarction; NSTEMI = non ST elevation myocardial infarction; AP = angina pectoris; UA = unstable angina; CAD = coronary artery disease.

Real-time polymerase chain reaction (RT-PCR) is the common technique used to extract microRNA in all involved studies. This systematic review concentrates mainly on cardiac-specific microRNAs, such as miR-1, miR-133a, and miR-208b, miR-499. In most available studies, these microRNAs sub-types were frequently observed to be up-regulated rapidly in the sample following myocardial necrosis. The majority of the studies further considered blood sampling timing once the symptoms started.

Four studies collectively investigated four miRNA, miR-1, miR-133a/b, miR-208a/b, and miR-499 [7,13,15,21]. Wang et al. in 2010 reported that each four microRNA levels were up-regulated, notably more increased than control ($p < 0.01$), and positively associated with cTnI. At the same time, the mean interval of the sampling of the blood was 4.8+3.5 hours [7]. Compared to other miRNAs, a more prominent diagnostic significance for AMI is demonstrated in miR-208a, having 0.965 (95% CI, 0.920-1.000) as the area-under-curve (AUC). However, the diagnostic significance of miR-208a was weaker than CTnI, which employs an AUC of 0.987 (95% CI, 0.966-1.000). In three AMI patients, they saw that the level of miR-208a was noticeable in one to four hours after the onset of chest pain, especially when the level of CTnI was identified to be under the cutoff value. Widera et al. in 2011 reported that compared to patients with unstable angina, patients with STEMI or NSTEMI were found to manifest with greater levels of miR-1, miR133a, and miR-208b [13]. miR-1, miR-133a, miR-133b, and miR-208b were found to be exclusively connected with CTnT levels in a multiple linear regression analysis which incorporated CTnT and clinical variables ($p < 0.001$). In 2011, Gidlof et al. published that all miRNA levels were considerably more eminent in diseased populations than in those who are normal ($p < 0.001$) [15]. Although other miRNAs did not associate with EF or CTnT, miR-208b was negatively correlated with ejection fraction (EF) and positively correlated with cTnT [15]. Samples of blood were retrieved at 24 hours, 48 hours, and 72 hours. Within 12 hours of the onset of the symptoms in STEMI patients, the levels of miR-1, miR-133a, miR-208b, and miR-499-5p were increased. Li YQ et al. in 2013 revealed that miR-1, -133a, -208b, and -499 levels were considerably elevated in samples of blood or plasma that are retrieved within 12 hours of the onset of AMI. However, for the diagnosis of AMI, the four results of increased miRNAs levels were not remarkable to CTnT ($p \geq 0.05$) [21].

As many as three studies investigated two microRNAs, miR-208b and miR-499, they found both upregulated [9,16,25]. In 2010, Corsten et al. identified a 1600-fold upregulation of miR-208b ($p < 0.005$) and a 100-fold higher level of miR-499 ($p < 0.0005$), and both biomarkers were significantly associated to CTnT ($p=0.0005$ in miR-208b and $p=0.0001$ in miR-499) when sampling time was less than 12 hours. In 2012, Devaux et al. recognized that the levels of miR-208b and miR-499 were significantly associated with CTnT and CK ($p < 10^{-9}$) with correlation coefficients of -0.18 ($p < 0.0008$) and inversely associated with the Ejection Fraction with correlation coefficients of -0.17 ($p < 0.001$). The study also found that miR-

208b and miR-499 levels were elevated in STEMI than NSTEMI ($p < 0.0001$) and elevated in AMI patients than in controls ($p < 0.0001$) [16]. Agiannitopoulos et al. in 2018 identified that miR-208b and miR-499 were significantly elevated in AMI patients than in control patients ($p < 0.0001$) and associated with CTnT ($p < 0.0001$) [25].

The other three studies that investigated miR-499 individually found the biomarker to be upregulated [8,22,23]. In 2010, Adachi et al. observed that miR-499 was associated with CK-MB and meaningfully elevated in the AMI group compared to the other groups ($p < 0.0001$). The blood sampling time was not beyond 48 hours after the onset of chest pain as a symptom [8]. The plasma concentration of miR-499 peaked at between six to 12 hours [8]. Chen et al. in 2015 published a study in which he took blood samples at 0 hours, 12 hours, 24 hours, three days, and seven days after the onset of chest pain [22]. The average duration of the onset of symptoms, which is chest pain, and the emergency room arrival was found to be 4.46±3.36 hours. Compared to those in unstable angina (UA) group and healthy control group, the relative plasma miR-499 level was meaningfully elevated in 53 patients with AMI (2.75±1.39 in UA group, 0.50±0.35 in the healthy control group, and 5.12±2.29 in AMI group). The discrepancies were statistically significant ($p < 0.01$) and positively-correlated with CTnI and CK-MB ($r=0.384$ vs $r=0.402$, $p < 0.01$ vs $p < 0.01$, respectively) [22]. In 2015, Zhao et al. stated that in MI group, miRNA-499 was meaningfully elevated compared to controls ($p < 0.05$) [23]. Three hours following the onset of chest pain in AMI, mRNA-499 could be identified in the serum, peaked after 12 hours, and progressively decreased after 15 hours [23]. In confirming AMI diagnosis, miR-499 (AUC of 0.915, 95% CI, 0.826-1.000) was inferior to CTnI (AUC of 0.971, 95% CI, 0.951-1.000) [23].

In two studies that examined miR-1, it was found that the biomarker is up-regulated [11,12]. In 2010, Ai et al. described that the level of miR-1 was remarkably elevated and positively associated with the cardiac troponin [11]. Cheng et al. in 2010 elaborated that the level of miR-1 was higher compared to healthy controls ($p < 0.05$) and positively correlated with the level of CK-MB ($r=0.68$; $p < 0.05$). The blood sampling average time was 8.5±3.82 hours [12].

In 2010, D'Alessandra et al. noticed the upregulation of miR-1, miR-133a, miR-133b, and miR-499-5p, and the downregulation of miR-122 and miR-375 [10]. The levels of miRNAs were remarkably altered as seen in the AMI group compared to the control group ($p < 0.01$). miRNAs were also found to be correlated positively with CTnI ($p < 0.01$). The average blood sampling time was 517+309 minutes following the onset of AMI. The plasma levels of miR-1, miR-133a, and miR-133b were formerly peaked at T0, or at a time point adjacent to the peak of CTnI. Reversely, miR-499-5p displayed a more gradual time progression and peaked behind the CTnI. In conclusion, the study summarized that after a three-day time development, the levels of miR-1, miR-133a, and miR-133b had recovered back to control levels.

Wang et al. in 2011 reported the upregulation of both miR-133 and miR-328 [6]. The 4.4-fold elevation of miR-133 in patients with AMI compared to the control group ($p=0.006$) in samples of whole blood was proportionate to plasma. The levels of miR-328 in patients with AMI were significantly elevated by 10.9-fold in plasma ($p=0.033$) and 16.1-fold whole blood compared to control ($p < 0.001$). The samples were

collected at T0 of 5.24±1.38 hours following AMI. There was an elevation of CTnI level at T0, stayed elevated till 20 hours, and was eventually returned to the normal amount seven days following the onset of symptoms. Nevertheless, the plasma or whole blood samples' levels of miR-133 and miR-328 were peaked at T0 already. The increase of miR-133 and miR-328 was reduced 20 hours following T0 and recovered to level of control seven days following T0. Still, the miR-133 and miR-328 displayed more rapid peaks compared to CTnI, and a positive association between miR-133 or miR-328 levels and the level of CTnI were also established.

Kuwabara et al. in 2011 inspected the upregulation of miR-1 ($p < 0.0005$) and miR-133a ($p < 0.0001$) [14]. A positive correlation with CTnT was also found in both miR-1 ($p < 0.005$) and miR-133a ($p < 0.0001$),

In 2012, Oerlemans et al. studied and elaborated the upregulation of miR-1 (OR of 1.44, 95% CI, 1.19-1.73), miR-208a (OR of 1.12, 95%CI, 0.95-1.35), miR-499 (OR of 1.38, 95% CI, 1.19-1.61), miR-21 (OR of 1.34, 95% CI, 1.15-1.55) and miR-146a (OR of 1.06, 95%CI, 0.97-1.15) [18]. miR-1, miR-499, and miR-21 was found to be superior compared to CTnT ($p<0.001$).

Gidlöf et al. in 2013 observed the upregulation of miR-1, miR-208b, and miR-499-5p [20]. miR-208b and miR-499-5p were markedly elevated in both patients with STEMI or NSTEMI compared to non-MI patients ($p < 0.001$). However, the result was inferior to CTnT, the modern gold standard cardiac marker. The average blood sampling time was 38.4 hours [20].

In 2013, Olivieri et al. came across that levels of plasma were upregulated in miR-1, miR-21, miR-133a, miR-208a, miR-423-5p and miR-499-5p [17]. Nonetheless, miR-499-5p displayed the greatest elevation contrasted with other miRNAs, all in NSTEMI versus control ($p <0.001$); NSTEMI versus congestive heart failure (CHF) ($p <0.05$), and CHF versus control ($p <0.05$). In the whole group, including the NSTEMI group, acute CHF group and control group, the level of miR-499-5p was markedly associated with CTnT ($p <0.001$) [17].

In 2013, Li et al. reported the upregulation of six miRNAs as opposed to the control. It was found in miRNAs as follow: miR-1, miR-223 and miR-499 ($p <0.05$), and miR-134, miR-186 and miR-208 ($p <0.001$) [19]. However, between these six miRNAs, only miR-208 and miR-499 have increased more prominent in patients with angina pectoris (AP) compared to patients with AMI. The values of AUC of the six-serum miRNAs as mentioned in the study were more prominent (0.830, 95% CI, 0.751-0.910) compared to the AUC of CTnT (0.768, 95% CI, 0.672-0.864) and the AUC of CK-MB (0.709; 95% CI, 0.606-0.812).

In a 2015 study conducted by Bialek et al., the level of miRNA-208a was elevated in patients with STEMI, especially during the admission time, and almost imperceptible in patients with CAD and the control group ($p <0.001$) [24]. The levels of miRNA-208a were heavily associated with the mass of CK-MB ($p <0.05$) and CTnI ($p <0.05$). The study also observed a notable elevation in miRNA-208a plasma level on the time of admission (time 0) in STEMI patients. The concentration of plasma miRNA-208a was elevated in the first three hours following the first symptoms and continued to elevate to 12 hours. The

study noted that the concentrations of CK-MB and CTnI mass were beneath the cutoff value for MI during admission time. Both biomarkers elevated at a later time, having peaked at six hours following the admission, and remained continued to elevate at the time of observation for around 48 hours.

Table 2. Summary of clinical studies which investigated cardiac-specific microRNAs in acute coronary syndrome populations

ıthor	miRNA Regulation	Source	Analysis Technique	Blood Sampling Timing	Results	Correlation with Biomarkers
et al.	Upregulated: miR-1, miR-133a, miR-499, miR-208a	Plasma	qRT-PCR	4.8±3.5 hours	miRNA levels were markedly elevated than control (p< 0.01)	miRNAs were correlated with cTnI
ıi et al.	Upregulated: miR-499	Plasma	qRT-PCR	48 hours	miR-499 values in the AM group were markedly elevated than other group (p<0.0001)	CK-MB
en et al.	Upregulated: miR-208a, miR-499	Plasma	PCR	<12 hours	miR-208b and miR-499 were markedly elevated (p<0.005 and p<0.0005, respectively) in AMI compared to control	miR-208b and miR-499 significantly correlated with CTnT
andra et)]	Upregulated: miR-1, miR-133a/b,miR-499-5p; Downregulated: miR-122, miR-375	Plasma	qRT-PCR	517+309 minutes	miRNAs levels were markedly altered in AMI compared to control (p<0.01)	Correlated positively with CTnI p<0.01 compared to control
ıl. [11]	Upregulated: miR-1	Plasma	PCR	Not given	miRNA levels were markedly elevated	Positive correlation with cardiac troponin
; et al.	Upregulated: miR-1	Serum	qRT-PCR	8.5±3.82 hours	miR-1 was elevated in AMI patients than in control group (p< 0.05)	miR-1 &CK-MB were correlated positively (r=0.68; p<0.05)

et al.	Upregulated: miR-133, miR-328	Whole blood Plasma	RT-PCR	5.24 ± 1.38 hours, 20 hours, 7 days	miR-133 plasma levels in AMI patients were elevated compared to control (p=0.006). miR-328 plasma and whole blood levels of AMI patients were markedly elevated compared to control (p=0.033 and p<0.001)	Correlated with CTnI
a et al.	Upregulated: miR-1, miR-133a; Insignificant: miR-133b, miR-208a, miR-208b, miR-499	Plasma	qRT-PCR	Not given	NSTEMI or STEMI patients presented with elevated levels of miR-1, miR133a, and miR-208b compared to unstable angina patients (p=0.001)	miR 133a (p<0.001), miR-1, miR-133a, miR-133b, and miR-208b were correlated independently with hsTnT levels (p<0.001)
bara et l]	Upregulated: miR-1, miR-133a	Serum	qRT-PCR	Not given	miR-1 p<0.0005, miR-133a p=0.001	miRNAs were correlated positively with cTnT
f et al.	Upregulated: miR-1, miR-133a, miR-208b,	Plasma	RT-PCR	24, 48, and 72	miR-1 (p<0.01); miR-	cTnT correlated positively with miR-208b (p=0.01, r 2=0.25);

	miR-499-5p			hours	133a ($p<0.01$); miR-208b ($p<0.001$); miR-499-5p ($p<0.01$) as compared to control	EF correlated negatively with miR-208b ($p=0.01$, $r^2=0.32$)
ıx et al.	Upregulated: miR-208b, miR-499	Plasma	RT-PCR	Not given	miRNAs were more elevated in MI patients ($p<0.001$)	Correlation of miRNAs and CK-MB and CTnT were highly significant $p<10^{-9}$. miRNAs were inversely correlated with the EF
ri et al.	Upregulated: miR-1, miR-21, miR-133a, miR-423-5p, miR-499-5p	Plasma	qRT-PCR	Not given	NSTEMI vs control $p<0.05$; NSTEMI vs CHF $p<0.05$; CHF vs CTR $p<0.05$	miR-499-5p and cTnT were correlated positively ($p<0.001$) in the total population and NSTEMI patients
ıan et 3]	Upregulated: miR-1, miR-208a, miR-499, miR-21, miR-146a	Serum	RT-PCR	Not given	Levels of circulating miRNAs were elevated in ACS patients. Circulating miR-21 and miR-146a levels markedly increased in ACS patients ($p<0.001$)	miRNA combined assay (miR-1, miR-499, and miR-21) was better than hs-CTnT
l. [19]	Upregulated: miR-1, miR-134, miR-186, miR-208, miR-223, miR-499	Serum	RT-PCR	Not given	Serum levels of the six miRNAs were increased in AMI than in control: miR-1, miR-223, and	Correlated to CKMB and CTnT

					miR-499 (p<0.05); miR-134, miR-186, miR-208 (p<0.001)	
f et al.	Upregulated: miR-1, miR-208b, miR-499-5p	Plasma	qRT-PCR	Average time to sample: 38.4 hours	miR-1 was elevated (p<0.01), miR-133a (p<0.01), miR-208b (p <0.001), and miR-499-5p (p<0.01) compared to healthy controls	miR-208b and miR -499-5p were correlated strongly with TnT. The accuracy was inferior to Troponin T. miR-1 was weakly associated with TnT
et al.	Upregulated: miR-1, miR-133a, miR-208b, miR-499	Plasma	qRT-PCR	Within 12 hours and day	All miRNAs were markedly elevated in AMI patients (p<0.001) than in healthy volunteers	miRNAs and cTnT were correlated positively. None of circulating miRNAs were better to cTnT for prompt diagnosis of AMI (p□0.05)
et al.	Upregulated: miR-499	Plasma	qRT-PCR	0, 12, 24, 72 hours and 7 days after AMI	miR-499 levels were markedly elevated immediately in AMI patients than in the UA and controls (p<0.01)	miR-499 correlated positively with CK-MB (p<0.01) and cTnI (p<0.01)
et al.	Upregulated: miR-499	Plasma	qRT-PCR	3, 12, and 15 hours	miRNA-499 in AMI was markedly elevated than in controls (p<0.05)	The sensitivity and specificity of microRNA-499 in AMI diagnosis were still inferior than cTnI
ɛ et al.	Upregulated: miR-208a	Serum	qPCR	0, 3, 6, 12, 24,	miR-208a was elevated in	miR-208a was related with CK-MB and CTnT mass

				and 48 hours	STEMI patients ($p<0.001$)	
nito-s et al.	Upregulated: miR-208b, miR-499	Plasma	PCR	Not given	miR-208b ($p<0.0001$), miR-499 ($p<0.0001$) compared to controls	All miRNAs were correlation with CTnT

Note: miR = microRNA; qRT = quantitative real time; PCR = polymerase chain reaction; RT-PCR = real time polymerase chain reaction; CTnI = cardiac troponin I; CTnT = cardiac troponin T; CK-MB = creatine kinase-muscle/brain; STEMI = ST elevation myocardial infarction; NSTEMI = non ST elevation myocardial infarction; hsTnT = high sensitive troponin T; AMI = acute myocardial infarction; UA = unstable angina; EF = ejection fraction; CHF = congestive heart failure.

Discussion

Lately, miRNAs are given immense importance in managing autophagy, necrosis, and apoptosis of cardiomyocytes, hence its function in myocardial infarction [26]. Investigations have additionally examined the pieces of evidence that miRNAs are seeped into the circulation from the heart following a myocardial injury, causing a dynamic increase in their levels [27,28]. Consequently, miRNAs which circulate in the plasma or blood have newly surfaced as promising biomarkers for the AMI diagnosis and/or AMI prognosis because of their reproducibility, specificity, and stability.

Our study reviews 20 articles which examine miRNAs in ACS patients. The enrolment criteria for patients with ACS or AMI in all investigations were standardized according to the international definitions of STEMI, NSTEMI, and unstable angina [29]. This review reveals the upregulated behavior of cardiac-specific miRNAs which were studied (miR-1, miR-133, miR-208, and miR-499) in an isolated or collective manner in each study, except in one study in which downregulation of two miRNAs, miR-122 and miR-375, were reported [10]. Aside from these miRNAs, several other upregulations occurred in miRNAs. Wang et al. reported miR-328, Oliveri et al. reported miR-423-5p, Oerleman et al. reported miR-21, and Li C et al. reported miR-223, miR-134 and miR-186 [6,17-19]. The microRNA retrieval sources were whole blood, serum, and plasma. miRNAs which circulate in the blood are stable. The real-time PCR assay was a well-known technique employed to quantify microRNA.

For the first time, Wang et al. in 2010 published a study that stated, among the four miRNAs investigated, the plasma levels observations of miR-208a could be implemented for AMI clinical diagnosis [7]. In terms of diagnosing AMI, miR-208a possessed greater specificity and sensitivity. miR-208a was readily identified in patients with AMI within four hours since the onset of chest pain. However, compared to CTnI, its diagnostic significance was inferior. No remarkable distinction in sex and age was mentioned in the study. Another study by Adachi et al. in 2010 revealed that plasma concentrations of miR-499 were increased in AMI patients within 48 hours [8]. The concentration was noted to peak in about six hours to

12 hours and later undetectable upon hospital discharge [8]. The upregulation of miR-499 was associated with the level of CK-MB.

Corsten et al. in 2010 found a notable rise in miR-208b and miR-499, correlating positively with the level of CTnI and CTnT [9]. The plasma levels of microRNAs were not influenced by a broad array of clinical confounding factors, which involve age, sex, systolic blood pressure, kidney function, body mass index, and white blood cell count. Another study by D'Alessandra et al. in 2010 presented an increased level of miR-1, miR-133a, miR-133b, and miR-499-5p and a decreased level miR-122 and miR-375, which are also positively correlated with CTnI [10]. It is unusual in a sense that the reduction in the concentration of miR-122 and miR-375 has not been examined in any medical condition, not in humans or the animal models of human diseases.

In 2010, Ai et al. revealed a remarkable elevation in miR-1 in patients with AMI, which also positively correlated with cardiac troponins [11]. An elevation in circulating miR-1 was not correlated with gender, age, blood pressure, diabetes mellitus state, and AMI biomarkers. Additionally, miR-1 has positively correlated also with the levels of CK-MB [12]. A remarkable elevation of miR-133a and miR-328 concentrations in patients with AMI and the correlation of CTnI with miR-133 or miR-328 concentrations were also confirmed by Wang et al. [6]. Nevertheless, the levels of miR-133 and miR-328 peaked faster compared to CTnI. No statistical deviations were reported between the control groups and the AMI groups for each of the analyzed variables, except for the levels of low-density lipoprotein (LDL) and total cholesterol (TC), which seemed to increase in AMI patients.

The upregulation of miR-1, miR-133a/b, miR-208a/b, and miR-499 levels and their correlation with CTnT was observed by Widera et al. [13]. miR-133a and miR-208b were correlated with all-cause mortality in six months, despite adjusting for sex and age. However, these miRNAs missed their correlation with the end results upon adjustment for hs-CTnT, which showed that, to a sensitive myonecrosis marker, these biomarkers do not contribute to prognostic information.

In 2011, Kuwabara et al. presented the upregulation of miR-1 and miR-133a and their positive correlation with the level of CTnT [14]. Levels of serum miR-1 and miR-133a were found to elevate in Takotsubo cardiomyopathy and UA but without an increase of cardiac troponins or CK concentrations in the serum.

Gidlof et al. in 2011 showed that, while other miRNAs did not associate with CTnT or EF, the upregulation of serum miR-1, miR-133a, miR-208b, miR-4995p, and miR-208b imposed positive correlation with CTnT and negative correlation with EF [15]. Even when patients with MI were distinguishable from patients of non-MI based on their miR-208b and miR-499-5p plasma levels, the precision was more inferior compared to CTnT, the modern gold standard of the cardiac marker.

In 2012, Devaux et al. noted that the concentration of miR-208b and miR-499 were more prominent in patients with MI since levels of plasma miRNAs rise starting from one hour after symptoms onset [16]. The peak concentration of CTnT and CK corresponded with the levels of both miRNAs. Additionally, there was an inverse relationship with EF, which mean that miRNAs may render data on the prognosis, despite

producing only a moderate left ventricular dysfunction prognosis. Another study by Olivieri et al. in 2013 revealed that miR-1, miR-21, miR-133a, miR-423-5p, and miR-499-5p levels were elevated in patients with NSTEMI compared to control. The concentration of miR-499-5p and miR-21 also a markedly elevated in NSTEMI patients compared to CHF [17]. Impressively, mir-499-5p was analogous to CTnT in distinguishing NSTEMI versus CHF and control patients. Its accuracy for diagnosis was more powerful than traditional hs-cTnT in distinguishing NSTEMI versus control. No meaningful impact of systemic arterial hypertension and type-2 diabetes mellitus was observed on the expression levels of miR-499-5p.

Oerlemans et al. in 2012 defined the inherent significance of miR-1, miR-21, miR-146a, miR-208a, and miR-499 in a group of 332 suspected patients with ACS. They noticed that, compared with hs-CTnT, the aggregate of miR-1, miR-21, and miR-499 have a greater diagnostic value [18]. The implementation of multivariate logistic regression was done to examine the independent predictability of ACS in miRNAs, after adjusting for important covariates, such as the history of the patient (sex, age, previous episode of MI, surgery or percutaneous intervention) and risk factors for cardiovascular diseases (hypercholesterolemia, hypertension, family history, diabetes mellitus, and smoking status).

In 2013, Li et al. measured levels of six serum miRNAs (miR-1, miR-134, miR-186, miR-208, miR-223 and miR-499) in patients with AMI and found that there was an upregulation in those patients versus control group [19]. For diagnosing AMI, measuring the assays of those six miRNAs is proved to be more solid predictive value than assessing a single miRNA individually. For the early and accurate diagnosis of AMI, collective assays of miRNAs can be used to supplement the cardiac troponins. All six miRNAs exhibited variances which are statistically significant between the AP and AMI. Moreover, the concentration of miR-208 and miR-499 were elevated more in AP than in AMI patients. The condition may be hinting that the miRNAs have a greater sensitivity for AP diagnosis. No significant variation in the gender, age, and ethnicity among the controls and the patients. In 2013, Gidlof et al. pointed that the concentrations of three miRNAs with cardio-enriched traits (miR-1, miR-208a, and miR-499-5p) were elevated in patients with NSTEMI compared to non-MI. They were foind to be elevated in patients with STEMI compared to NSTEMI, although, the precision was more inferior than CTnT, the modern gold standard of the cardiac marker [20]. A multivariate analysis was done, and the statistical significance level for all associations was not influenced despite adjustment for sex, age, and sampling time.

The levels of miRNA-1, miRNA-133a, miRNA-208b, and miRNA-499 were found to be elevated significantly in post-AMI patients compared to normal volunteers who were paired for sex and age [21]. Even though positive correlations were observed between cTnT and the four circulating miRNAs in 12 hours after the onset of the symptoms, for prompt diagnosis of AMI, no miRNAs was reported to be superior to CTnT.

The level of serum miR-1 rose swiftly hours after symptoms of AMI [12]. Over 20-fold increase in the level of serum miR-1 was detected within 24 hours of AMI. Furthermore, there was a positive correlation which links CK-MB and serum miR-1. The report also proposed that miR-1 may additionally be associated with the size of myocardial infarction in humans. Chen et al. have also pointed out that plasma levels of miR-499 markedly elevated 12 hours after the onset in patients with AMI, and imposed positive correlation

with cardiac biomarkers [22]. Remarkably, they noticed that miR-499 concentration in two- and three-vessel CAD was considerably greater than single-vessel CAD; therefore, the biomarker corresponds positively with coronary stenosis severity. miR-499 levels in patients with AMI in 24 hours following an emergency percutaneous intervention (PCI) were notably more profound than the non-PCI group and those at admission.

Zhao et al. reported that miR-499 concentration in patients with AMI was markedly more elevated than the control group, despite having moderate specificity and sensitivity than CTnI [23]. The half-life of plasma miR-499 is low; its levels markedly elevate in three hours following the AMI onset, peaked at 12 hours, before progressively decreasing. We anticipate that this conclusion would be instrumental for re-infarction diagnosis following an initial AMI. No notable variation in gender and age within the groups.

As explained by Bialek et al., miR-208a, exclusively synthesized in the heart, rises in conditions such as STEMI and/or myocardial injury induced by reperfusion [24]. During admission (less than three hours of the onset) in STEMI patients when CTnI was not increased yet, miR-208a concentration in plasma elevated to 10-fold increase. miRNA-208a also peaked in advance of both CK-MB and CTnI mass. This condition exhibits a favorable relationship with the traditional biomarkers of myocardial injury.

Lastly, Agiannitopoulos et al. reported a study of Greek AMI patients where the upregulation of miR-208b and miR-499 occurred [25]. An immediate collection of blood samples were performed once the patient is being admitted. Interestingly, the results of the Greek AMI group are concomitant with those from Asian groups, implying that the genetic background does not influence the expression levels of miRNA-208b and miRNA-499. No remarkable distinctions were observed between both groups, particularly in gender, age, smoking condition, and any additional clinicopathological characteristics.

LIMITATIONS

Multiple limitations developed during the development of this study. First, not every study cohort was gender- and age-matched or matched with additional cofounding factors. Second, the population size of some trials was relatively modest. Third, the timing for retrieving blood samples was not discussed in several studies, despite having significance to faithfully label microRNA as a useful cardiac biomarker in the prompt diagnosis of AMI. Fourth, microRNA levels should be investigated in CKD patients to eliminate false positive outcomes. There is a need to carry extensive randomized cohort studies to approach these limitations and assess the potential advantages of having microRNAs as a novel cardiac biomarker.

Conclusions

Cardio-specific microRNAs (miR-1, miR-133, miR-208, and miR-499) are potent novel biomarkers to perform timely diagnosis and prognosis of acute myocardial infarction. Six serum miRNAs (miR-1, miR-134, miR-186, miR-208, miR-223 and miR-499) posed a greater sensitivity for the diagnosis of angina pectoris. The cardio-specificity of these microRNAs correlates strongly with the conventional cardiac biomarkers and the time when their concentration levels in the blood elevated. Studies involving miR-

208b and miR-499 concluded that ethnic and genetic background does not affect the increasing level of miRNAs, the level of both biomarkers correspond positively with the severity of coronary artery stenosis, and that miRNAs may render data on the prognosis of the disease. In acute myocardial infarction cases, the levels of miRNAs rise ahead of the conventional cardiac biomarkers. The reduction of particular miRNAs (miR-122 and miR-375) was detected only in acute myocardial infarction and has not been found in other medical conditions—neither in humans nor animal models. Therefore, additional investigations at greater measure are required to assess the promising role of having microRNA as the novel cardiac biomarker and portray their performance in enhancing the diagnostic strategy in patients with acute coronary syndrome.

Declarations

Funding

None.

Conflict of Interest

The author declares there is no conflict of interest regarding all aspect of the study.

Author Contribution

PHW is responsible for the study from the literature search, data gathering, data analysis, until reporting the results of the study.

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References

1. Abubakar II, Tillmann T, Banerjee A:Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015, 10:117-71. 1016/S0140-6736(14)61682-2
2. Acute coronary syndrome. (2018). Accessed: December 9, 2020:https://emedicine.medscape.com/article/1910735.
3. Freda BJ, Tang WW, Van Lente F, Peacock WF, Francis GS:Cardiac troponins in renal insufficiency: review and clinical implications. *J Am Coll Cardiol*. 2002, 18:2065-71. 1016/S0735-1097(02)02608-6
4. Hammond SM:An overview of microRNAs. *Adv Drug Deliv Rev*. 2015, 29:3-14. 1016/j.addr.2015.05.001
5. Schulte C, Zeller T:microRNA-based diagnostics and therapy in cardiovascular disease—summing up the facts. *Cardiovasc Diagn Ther*. 2015, 5:17. 3978/j.issn.2223-3652.2014.12.03

6. Wang R, Li N, Zhang Y, Ran Y, Pu J:Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. *Intern Med*. 2011, 50:1789-95. 2169/internalmedicine.50.5129
7. Wang GK, Zhu JQ, Zhang JT, et al.:Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur Heart J*. 2010, 16:659-66. 1093/eurheartj/ehq013
8. Adachi T, Nakanishi M, Otsuka Y, et al.:Plasma microRNA 499 as a biomarker of acute myocardial infarction. *Clin Chem*. 2010, 56:1183-5. 1373/clinchem.2010.144121
9. Corsten MF, Dennert R, Jochems S, et al.:Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease. *Circ Cardiovasc Genet*. 2010, 3:499-506. 1161/CIRCGENETICS.110.957415
10. D'alessandra Y, Devanna P, Limana F, et al.:Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. *Eur Heart* 2010, 9:2765-73. 10.1093/eurheartj/ehq167
11. Ai J, Zhang R, Li Y, et al.:Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. *Biochem Biophys Res Commun*. 2010, 391:73-7. 1016/j.bbrc.2009.11.005
12. Cheng Y, Tan N, Yang J, et al.:A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. *Clin Sci (Lond)*. 2010, 119:87-95. 1042/CS20090645
13. Widera C, Gupta SK, Lorenzen JM, et al.:Diagnostic and prognostic impact of six circulating microRNAs in acute coronary syndrome. *J Mol Cell Cardiol*. 2011, 51:872-5. 1016/j.yjmcc.2011.07.011
14. Kuwabara Y, Ono K, Horie T, et al.:Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. *Circ Cardiovasc Genet*. 2011, 4:446-54. 1161/CIRCGENETICS.110.958975
15. Gidlöf O, Andersson P, Van Der Pals J, Götberg M, Erlinge D:Cardiospecific microRNA plasma levels correlate with troponin and cardiac function in patients with ST elevation myocardial infarction, are selectively dependent on renal elimination, and can be detected in urine samples. *Cardiology*. 2011, 118:217-26. 1159/000328869
16. Devaux Y, Vausort M, Goretti E, et al.:Use of circulating microRNAs to diagnose acute myocardial infarction. *Clin Chem*. 2012, 58:559-67. 1373/clinchem.2011.173823
17. Olivieri F, Antonicelli R, Lorenzi M, et al.:Diagnostic potential of circulating miR-499-5p in elderly patients with acute non ST-elevation myocardial infarction. *Int J Cardiol*. 2013, 31:531-6. 1016/j.ijcard.2012.01.075
18. Oerlemans MI, Mosterd A, Dekker MS, et al.:Early assessment of acute coronary syndromes in the emergency department: the potential diagnostic value of circulating microRNAs. *EMBO Mol Med*. 2012, 4:1176-85. 1002/emmm.201201749
19. Li C, Fang Z, Jiang T, Zhang Q, Liu C, Zhang C, Xiang Y:Serum microRNAs profile from genome-wide serves as a fingerprint for diagnosis of acute myocardial infarction and angina pectoris. *BMC Med Genomics*. 2013, 6:16-10. 1186/1755-8794-6-16

20. Gidlöf O, Smith JG, Miyazu K, Gilje P, Spencer A, Blomquist S, Erlinge D:Circulating cardio-enriched microRNAs are associated with long-term prognosis following myocardial infarction. *BMC Cardiovasc Disord*. 2013, 13:12-10. 1186/1471-2261-13-12
21. Li YQ, Zhang MF, Wen HY, et al.:Comparing the diagnostic values of circulating microRNAs and cardiac troponin T in patients with acute myocardial infarction. *Clinics*. 2013, 68:75-80. 6061/clinics/2013(01)OA12
22. Chen X, Zhang L, Su T, et al.:Kinetics of plasma microRNA-499 expression in acute myocardial infarction. *J Thorac Dis*. 2015, 7:890. 3978/j.issn.2072-1439.2014.11.32
23. Zhao CH, Cheng GC, He RL, Hong Y, Wan QL, Wang ZZ, Pan ZY:Analysis and clinical significance of microRNA-499 expression levels in serum of patients with acute myocardial infarction. *Genet Mol Res*. 2015, 27:4027-34. 4238/2015.April.27.17
24. Białek S, Górko D, Zajkowska A, et al.:Release kinetics of circulating miRNA-208a in the early phase of myocardial infarction. *Kardiol Pol*. 2015, 73:613-9. 5603/KP.a2015.0067
25. Agiannitopoulos K, Pavlopoulou P, Tsamis K, et al.:Expression of miR-208b and miR-499 in Greek patients with acute myocardial infarction. *In Vivo*. 2018, 32:313-8. 21873/invivo.11239
26. Sun T, Dong YH, Du W, et al.:The role of microRNAs in myocardial infarction: from molecular mechanism to clinical application. *Int J Mol Sci*. 2017, 18:745-10. 3390/ijms18040745
27. Akat KM, Moore-McGriff DV, Morozov P, et al.:Comparative RNA-sequencing analysis of myocardial and circulating small RNAs in human heart failure and their utility as biomarkers. *Proc Natl Acad Sci USA*. 2014, 29:11151-6. 1073/pnas.1401724111
28. De Rosa S, Fichtlscherer S, Lehmann R, Assmus B, Dimmeler S, Zeiher AM:Transcoronary concentration gradients of circulating microRNAs. *Circulation*. 2011, 124:1936-44. 1161/CIRCULATIONAHA.111.037572
29. Acute coronary syndrome: terminology and classification. (2018). Accessed: December 12, 2020:https://www.uptodate.com/contents/acute-coronary-syndrome-terminology-and-classification.