


## REVIEW ARTICLE

# Circulating microRNAs as predictive biomarkers of coronary artery diseases in type 2 diabetes patients

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## Abstract

**Background:** Type 2 diabetes mellitus (T2DM) is an increasing metabolic disorder mostly resulting from unhealthy lifestyles. T2DM patients are prone to develop heart conditions such as coronary artery disease (CAD) which is a major cause of death in the world. Most clinical symptoms emerge at the advanced stages of CAD; therefore, establishing new biomarkers detectable in the early stages of the disease is crucial to enhance the efficiency of treatment. Recently, a significant body of evidence has shown alteration in miRNA levels associate with dysregulated gene expression occurring in T2DM and CAD, highlighting significance of circulating miRNAs in early detection of CAD arising from T2DM. Therefore, it seems crucial to establish a link between the miRNAs prognosing value and development of CAD in T2DM.

**Aim:** This study provides an overview on the alterations of the circulatory miRNAs in T2DM and various CADs and consider the potentials of miRNAs as biomarkers prognosing CADs in T2DM patients.

**Materials and Methods:** Literature search was conducted for miRNAs involved in development of T2DM and CAD using the following key words: "miRNAs", "Biomarker", "Diabetes Mellitus Type 2 (T2DM)", "coronary artery diseases (CAD)". Articles written in the English language.

**Result:** There has been shown a rise in miR-375, miR-9, miR-30a-5p, miR-150, miR-9, miR-29a, miR-30d, miR-34a, miR-124a, miR-146a, miR-27a, and miR-320a in T2DM; whereas, miR-126, miR-21, miR-103, miR-28-3p, miR-15a, miR-145, miR-375, miR-223 have been shown to decrease. In addition to T2DM, some miRNAs such as miR-1, miR-122, miR-132, and miR-133 play a part in development of subclinical aortic atherosclerosis associated with metabolic syndrome. Some miRNAs increase in both T2DM and CAD such as miR-1, miR-132, miR-133, and miR-373-3-p. More interestingly, some of these miRNAs such as miR-92a elevate years before emerging CAD in T2DM.

**Conclusion:** dysregulation of miRNAs plays outstanding roles in development of T2DM and CAD. Also, elevation of some miRNAs such as miR-92a in T2DM patients can efficiently prognose development of CAD in these patients, so these miRNAs can be used as biomarkers in this regard.

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## KEYWORDS

biomarker, coronary artery diseases (CAD), diabetes mellitus type 2 (T2DM), microRNAs

## 1 | INTRODUCTION

About half a billion people across the globe are affected by diabetes. This disease predisposes a significant number of people to life-threatening complications such as heart conditions and other diabetes-related morbidities.<sup>1</sup>

Sedentary lifestyles, obesity, aging, and urbanization are the contributing factors for type 2 diabetes mellitus (T2DM). Additionally, hereditary backgrounds are involved in the etiology of the disease.<sup>2</sup> T2DM is characterized by chronic hyperglycemia and faulty metabolism of carbohydrates, lipids, and proteins, resulting from insulin resistance and insufficient insulin secretion.<sup>3</sup> Regarding dysregulated metabolism of lipids, patients with T2DM are highly susceptible to cardiovascular diseases, particularly coronary syndrome.<sup>4</sup>

Acute coronary syndrome (ACS) is an expression to describe three types of coronary artery disease due to the dissociation of plaques from the lumen of the coronary artery. The classification is based on the location of the blockage, duration of blockage, and severity of damage. These life-threatening conditions require urgent medical intervention. The three types are unstable angina, non-ST segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI). Unstable Angina is the acute form of coronary syndrome and can simply turn into myocardial infarction. As a result, it is treated as a medical emergency. Non-ST segment elevation myocardial infarction (NSTEMI) may not cause changes on an electrocardiogram (ECG). In addition, the blockage may be partial or temporary, and so the extent of the damage is relatively small. However, ST-segment elevation myocardial infarction (STEMI) arises from the prolonged blocked blood supply.<sup>5</sup>

Establishing simple and valid clinical biomarkers is crucial in detecting and treatment of different coronary artery diseases (CAD) and T2DM. microRNAs (miRNAs) can be used as biomarkers which are highly stable in circulation and specific to disease and organ; thus, they have potential to be applied as reliable diagnostic biomarkers.<sup>6</sup>

miRNAs are stable single-stranded RNAs modulating various biological processes by regulating gene expression via binding to the 3'-untranslated regions of target mRNAs at the post-transcriptional level.<sup>7</sup> They are primarily located within the introns of the host genes and are transcribed by RNA polymerase II.<sup>8,9</sup>

The human genome encodes roughly 1000 miRNAs, more than 100 of them have been identified in the serum of healthy subjects.

Unlike intracellular mRNAs, circulating miRNAs are significantly resistant against degradation by RNases. These miRNAs may also be produced by blood cells or tissues like heart, lung, liver, and kidney.<sup>6</sup> They are mostly preserved in macrovesicles such as exosomes, microparticles, and apoptotic bodies, possibly protecting them against degradation by RNases. Their stability and source specificity make miRNAs as reliable candidates in detecting diseases like CAD and T2DM.<sup>6,10</sup>

## 2 | ROLES OF miRNAs IN T2DM DEVELOPMENT

Dysregulation of miRNAs has been demonstrated widely in T2DM (see Table 1). There is a rise in, miR-9, miR-30a-5p, miR-150, miR-9, miR-29a, miR-30d, miR-34a, miR-124a, miR-146a, miR-27a, and miR-320a in T2DM. On the contrary, other miRNAs such as miR-126, miR-21, miR-103, miR-28-3p, miR-15a, miR-145, miR-375, and miR-223 were shown to be decreased.<sup>11,12</sup>

Kong et al. studied the expression pattern of several miRNAs associated with the pathogenesis of T2DM and compared the results among the recently diagnosed cases of T2DM, pre-T2DM subjects, and T2DM-susceptible subjects with normal glucose tolerance.<sup>13</sup> They showed the levels of miRNAs were higher in T2DM patients compared to T2DM-susceptible subjects. Additionally, the expressions of some miRNAs were significantly lower in the pre-T2DM individuals in comparison with T2DM patients. This study also demonstrated the role of miR-29a, miR-9, miR-30d, miR-124a, miR-146a, miR-34a, and miR-375 in fine-tuning insulin secretion and pathogenesis of T2DM, while their expression levels remained steady in the pre-T2DM stage, refusing their potential relevance as disease-specific markers.<sup>14</sup>

The role of miR-146a in the inflammation and insulin resistance was shown to be more significant in patients with T2DM than in those with normal glucose tolerance (NGT). There was a significant lower expression level of miR-146a in T2DM subjects.<sup>15</sup>

Further association between miRNAs and metabolic syndrome was demonstrated; miRNA-150, miR-192, miR-27a, miR-320a, and miR-375 were upregulated in T2DM, highlighting their part in the regulation of hyperglycemia. This finding made great strides in introducing the clinical application of these miRNAs in the risk assessment of T2DM and metabolic syndrome.<sup>16</sup>

TABLE 1 Changes in the levels of miRNAs in T2DM

Upregulation	Downregulation
miR-9, <sup>17</sup> miR-30a-5p, <sup>19</sup> miR-150, <sup>20</sup> miR-29a, <sup>21</sup> miR-30d, <sup>14</sup> miR-34a, <sup>22</sup> miR-124a, <sup>23</sup> miR-146a, <sup>15</sup> miR-27a, <sup>23</sup> miR-320a <sup>24</sup>	miR-126, <sup>24</sup> miR-21, <sup>25</sup> miR-103, <sup>26</sup> miR-28-3p z24), miR-15a, <sup>27</sup> miR-145, <sup>28</sup> miR-375, <sup>29</sup> miR-223 <sup>30</sup>

TABLE 2 Roles of CAD-related miRNAs in diagnosis, severity, and prognosis of heart diseases

Disease	miRNA alterations		Diagnosis		Ref.
	Increase	Decrease	Increase	decrease	
Unstable angina	miR-135 and miR147	miR-135, 147	miR-122-5p, 223-3p, 941	miR-378, 196-5p, 3163-3p	49-52
Acute MI	miR-1, 122, 126-3p, 30c, 26a, miR-22-5p and miR-150-3p	miR-15 family, 195, miR-590-3p, miR-199a-3p, miR-132-5p	miR-223	miR-378, 196-5p, 3163-3p	17,18,53,54
NSTEMI	miR-221-3p, 483-5p		miR-223	miR-378, 196-5p, 3163-3p	53,55
STEMI	miR-221-3p, 374b-5p		miR-142-3p, miR-17-5p, 499-a-5p, 223	miR-378, 196-5p, 3163-3p	35,56,57
HF	miR-499, 423-5p, 21, 132, 122, 210, 499, 622	miR-150-5p	miR-223	miR-378, 196-5p, 3163-3p	58-60
Arrhythmia	miR-1443p, 145-5p, 185-5p, 494	miR-23a and miR-26a	miR-223	miR-378, 196-5p, 3163-3p	58,60,61
Atherosclerosis	miR-133, 208a, 146b, 34a, 21, 30a, 106b, 133a, 208a, 155, 223	miR-31, 720, 126-5p, 17-92	miR-223	miR-378, 196-5p, 3163-3p	62-64

Abbreviations: HF, Heart Failure; MI, Myocardial Infarction; NSTEMI, non-ST-segment elevation myocardial infarction; STEMI, ST-elevated myocardial infarction.

Zhang et al. assessed the miRNA in individuals with normal blood glucose, individuals susceptible to developing T2DM, and T2DM patients; they showed only miR-15a, miR-223, and miR-126 were detectable in T2DM patients. A significant finding was that the plasma miR-126 levels were similar between pre-T2DM and T2DM groups, while, it was substantially lower in the NGT group. Furthermore, the serum levels of miR-126 were shown to be associated with levels of fasting glucose in the serum. This study suggested that the plasma miR-126 could be regarded as a biomarker for the non-invasive prediction and diagnosis of T2DM.<sup>17</sup>

Stepien et al. analyzed the angiogenic capacity of ectosome-derived miRNAs among T2DM patients. They reported that miR-221-3p, miR-20a-3p, let-7i-5p miR-26a-5p, miR-26b-5p, miR-30b-5p miR-29a-5p, miR-374a-5p, miR-30c-5p, and miR-199a-3p were significantly elevated in ectosomes obtained from T2DM patients [57]. Also, they showed that the expression levels of miR-193b-3p and miR-95-3p in the ectosomes-enriched plasma were notably higher in T2DM, while the expression of miR-409-3p was lower in T2DM. These findings may highlight the role of miRNAs in dysregulated angiogenesis and development of vascular complications in patients with T2DM.<sup>18</sup> Table 1 shows the status of different miRNAs in T2DM.

### 3 | ROLES OF miRNA IN CAD

Micro-RNAs play a critical role in cardiac development and pathological processes such as AMI (including NSTEMI and STEMI) and other cardiovascular diseases such as arrhythmias, hypertrophy, heart failure, and atherosclerosis.<sup>31</sup> More than 200 miRNAs have been identified in the heart tissues. miRNAs such as miR-1, let-7, miR-133, miR-126-3p, miR-30c, and miR-26a were found to be prevalent in the cardiac muscles and miR-145, let-7, miR-125b, miR-125a, miR-23, and miR-143 in the arterial smooth muscles.<sup>32,33</sup> Additionally, miR-122 and miR-1 have been introduced as myocardium-specific markers.<sup>34</sup>

Alterations in some miRNAs can be used as diagnostic biomarkers for coronary artery diseases, such as the downregulation of miR-378, 196-5p, and 3163-3p, which can assist in distinguishing CAD patients from normal subjects.<sup>35,36</sup> While upregulation of miR-223 shows the occurrence of CAD.<sup>37</sup>

There has been shown a reduction in the miR-15 family in MI which can be used in diagnosis of T2DM patients and pre-diabetic ones.<sup>38</sup> In addition, several miRNAs in patients with ST-segment elevation MI, miR-142-3p and miR-17-5p could be considered as potential diagnostic markers.<sup>39</sup>

The prognostic value of miR-1254 was shown. This micro-RNA correlates with the left ventricular remodeling and systolic function.<sup>39</sup>

Also, circulating miR-22-5p and miR-150-3p were overexpressed in the early stage of acute MI. On the other hand, miR-132-5p was reduced in patients with acute MI.<sup>40,41</sup>

Moreover, miR-499 has previously been used in the diagnosis of AMI and NSTEMI whereas miR-423 was used in the diagnosis of AMI

TABLE 3 The role of miRNAs in metabolic syndrome in T2DM and CAD

miRNAs as T2DM biomarkers	Glucose metabolism	Inflammation	Platelet reactivity	Endothelial dysfunction	Overlapped change in miRNAs in both CAD and T2DM
Upregulation	miR-124a, <sup>23</sup> 150, <sup>83</sup> 27a, <sup>23</sup> 30a-5p, <sup>19</sup> 30d, <sup>21</sup> 320a, <sup>16</sup> 34a, <sup>16</sup> 146a <sup>84</sup>	miR-146a <sup>85</sup>			miR-1, <sup>86-88</sup> miR-132, miR-133, <sup>86</sup> miR-373-3-p <sup>89</sup>
Downregulation	miR-375, <sup>90</sup> 91, 29a, <sup>92</sup> 103, <sup>26</sup> 21, <sup>93</sup> 28-3p, <sup>24</sup> 223, <sup>30</sup> 145, <sup>28</sup> 15a, <sup>27</sup> 126 <sup>24</sup>	miR-103, <sup>26</sup> 21-5p, <sup>58</sup> 126-3p <sup>94</sup>	miR-223, <sup>95</sup> 140, <sup>63</sup> 26b, <sup>126</sup> 95	miR-21-5p, <sup>58</sup> 126-3p, 26a, <sup>96</sup> 24 <sup>61</sup>	

and STEMI.<sup>42</sup> Early detection of AMI has been facilitated via measuring miR-208 only one hour after infarction. However, its levels fell after three hours. In such a case, assessment of other markers including miR-499 and miR-133 is crucial.<sup>43</sup>

The analysis of patients with both stable CAD and acute coronary syndrome showed upregulation of miR-92a-3p and miR-206<sup>44,45</sup>; while reduced levels were observed in miR-939 and miR-181-a-3p and 181-a-5p.<sup>13,46</sup>

#### 4 | miRNAs RELEASED BY VASCULAR AND BLOOD CELLS CAN BE USED AS BIOMARKERS CAD DEVELOPMENT

CAD stems from atherosclerosis, an inflammatory disease of the arteries characterized by the formation of atherosclerotic plaques. These plaques cause the narrowing of the lumen of the artery, which can lead to thrombus formation and eventually complete obstruction of the lumen. All the cellular components involved in atherosclerosis are susceptible to release miRNAs into the bloodstream. On the other hand, some specific miRNAs may be taken up by the cells involved in atherosclerosis. In both conditions, miRNAs can be considered as diagnostic biomarkers of atherosclerosis.<sup>47</sup> Various miRNA including miR-181d, miR-140-3p, miR-182, miR-145, miR-19a, miR-584, miR-155, miR-222, miR-29a, miR-378, miR-342, miR-150, and miR-30e-5p in whole blood,<sup>48</sup> and miR-126, miR-92a, miR-145, miR-17, and miR-155 in in EDTA (ethylenediaminetetraacetic acid) plasma and serum have been shown as the biomarkers of CAD.<sup>47,48</sup> The prognostic and diagnostic value of miRNAs is shown in Table 2.

Fichtlscherer et al. measured 8 miRNAs in patients with CAD; the endothelial enriched miRNAs including miR-126, miR-17, and miR-92a, the smooth-muscle cell-enriched miR-145, and the inflammatory cell-enriched miR-155 were significantly reduced, whereas the cardiomyocyte enriched miRNAs miR-133 and miR-208a were elevated.<sup>65</sup> Also, elevations in miR-146, miR-19, miR-155, miR-21, as well as miR-223 were shown in patients with ACS compared to patients with CAD.<sup>66</sup> Afterward, Li et al. identified miR-130a, miR-21, miR-27b, and miR-210 as potential biomarkers for peripheral artery disease. The promising finding of this study shows a lack of significant overlap between the elevated miRNAs in coronary and peripheral atherosclerosis. Accordingly, this finding may indicate the tissue-specificity of these miRNAs.<sup>67</sup>

Taurino et al. assessed the potential of circulating miRNAs in whole blood as biomarkers for CAD; they showed an elevation in the levels of miR-140-3p and miR-182 in CAD patients.<sup>48</sup> Also, lower levels of miR-19a, miR-181d, miR-222, miR-342, miR-484, miR-155, miR-145, miR-29a, miR-378, miR-150, and miR-30e-5p were shown in CAD patients.<sup>61,65</sup>

Peripheral blood mononuclear cells have been shown to release miRNA biomarkers specific to acute coronary syndromes and unstable angina. The miRNA signature was studied by Hoekstra et al. in PBMCs of patients with CAD; they revealed that miR-135 and miR147 were downregulated in patients with stable

and unstable angina pectoris (AP). Also, they showed that three miRNAs (miR-134, miR-370, and miR-198) were remarkably up-regulated in the PMNCs of patients with unstable AP compared with stable AP, suggesting that the expression of these miRNAs in PMNCs could be regarded as a risk factor of developing acute coronary syndromes.<sup>68</sup> Furthermore, Takahashi et al. showed the elevated levels of the miRNAs associated with inflammation (miR-146a and miR-146b) in PBMCs of stable patients with CAD. After 12 months, 19 percent of the patients experienced a cardiac event; thereby, miR146a level turned out to be a useful independent prognostic marker of these events. Furthermore, in circulating endothelial progenitor cells, miR-221 and miR-222 were found to be elevated in patients with CAD compared to those with no indication of CAD.<sup>69</sup>

## 5 | microRNA IN T2DM PREDICTING CAD

Diabetic heart disease (DHD) is defined as the heart disease in diabetic individuals including CAD, heart failure, and/or cardiomyopathy.<sup>70</sup> It mostly arises from obesity, physical inactivity, advanced age, and metabolic syndrome.<sup>71-75</sup> In a study performed by Ramzan et al., miR-15a-5p, miR-17-5p, miR-370-3p, and miR-375 significantly predicted metabolic syndrome. Analysis of predictive miRNAs showed miR-15a-5p and miR-17-5p. were involved in the regulation of metabolic pathways, including insulin, *wnt*, fatty acid metabolism, and AMPK.<sup>76</sup>

A study of the expression of miR-126 and miR-26a showed significant differences between patients with and without T2DM. Both miRNAs were downregulated in T2DM patients. Interestingly, patients with less miR-26a and miR-126 were more prone to develop subsequent CAD. Also, miR-24 was shown to reduce in T2DM-CAD patients, while the mRNA of YKL-40, an inflammatory mediator involved in endothelial dysfunction, was elevated in both T2DM-CAD and CAD patients.<sup>77</sup> Furthermore, it was reported that the reduced levels of miR-145, miR-9, miR-15a, miR-103, miR-28-3p, miR-29a, miR-223, miR-126, and miR-375 are reliable predictors of CAD patients in type 2 diabetes. miR-24 and its target chitinase 3-like 1 (Chi3l1/YKL40) were also reduced in type 2 diabetes patients with CAD.<sup>77,78</sup> Additionally, hyperglycemic-induced alterations in miRNA in patients with diabetic heart issues are likely to be irreversible even after glycemic control.<sup>79</sup>

The levels of miR-92a in diabetes patients were shown to precede the emerging of acute coronary at least 2 years before. This study showed that these T2DM patients had a significant increase in the levels of miR-92a compared to coronary heart disease. Also, this study indicated that miR-92a is associated with the coincidence of diabetes and heart disease, as well as high blood pressure and HbA1c.<sup>80</sup> It is also implicated that miR-133a and miR-373 mediate signaling of myocytes enhancer factor2C (MEF2C) in diabetic cardiomyopathy, which is an essential transcription factor underlying myocardial hypertrophy and cardiac fibrosis.<sup>81,82</sup>

Table 3 shows miRNAs involved in metabolic syndrome with similar roles in T2DM and CAD.

## 6 | CLINICAL PERSPECTIVE AND CONCLUSION

Since their identification, cardiovascular miRNAs have always been regarded as key players in regulating cardiac gene expression under normal and pathological conditions.<sup>97</sup> Since T2DM patients are highly susceptible to develop CAD, so establishing reliable biomarkers to prognosis CAD in these patients can play a significant part in mitigation T2DM burden.

Dysregulation of miRNAs occurs in T2DM and CAD and underlying pathological events in both situations (see Tables 1,2, and 3). A pile of studies has shown that miR-1, miR-132, miR-133, and miR-373-3-p increase in both T2DM and CAD (see Table 3). Some of these miRNAs emerge long before cardiac events. For instance, the levels of miR-92a in acute coronary patients were shown to be preceded by diabetes for at least two years.<sup>80</sup> Accordingly, these miRNAs may assist in following up T2DM patients who are prone to develop CAD.

Regarding the Role of miRNAs in atherosclerosis which is the primary cause of CAD, it is reported that microRNAs participate either beneficially or harmfully in almost all molecular pathways of atherosclerosis and arterial remodeling, including endothelial dysfunction, monocyte activation, arterial wall invasion, platelet, and vascular smooth muscle cell activation.<sup>98</sup> Atherosclerosis has been regarded as a disease of chronic inflammation.<sup>99,100</sup> Therefore, miRNA may play their part in heart-related diseases through induction of inflammation. miR-92a has been shown to contribute to cardiovascular disease development in diabetes mellitus through NF- $\kappa$ B and downstream inflammatory pathways.<sup>101</sup> Also, miR-132 was shown to inhibit the expression of SIRT1 and induce the pro-inflammatory processes of vascular endothelial inflammation through blocking the SREBP-1c metabolic pathway.<sup>102</sup> miR-1, miR-122, miR-132, and miR-133 are related to subclinical aortic atherosclerosis associated with metabolic syndrome.<sup>103</sup> Therefore, these miRNAs may strongly prognose the development of CAD in T2DM patients.

Along with miRNAs, the inflammatory biomarkers can be used as predictors of severity and prognosis in CAD in T2DM patients to stratify the risk of these patients, to take the best therapeutic approach, and predict the results after interventions.<sup>99</sup>

Some limitations should be regarded in future studies. Most studies have evaluated miRNAs in populations of fewer than 100 subjects, so bigger sample sizes seem to be crucial in future studies. Also, the value of the miRNAs in the prognosis of the diseases, such as the risk of MI, should be assessed. Furthermore, it would clarify whether miRNAs levels are practical tools to evaluate the response to therapy.<sup>104</sup> Also, low levels of total RNA in plasma or serum, which make the miRNA amplification often necessary to measure circulating miRNAs. U6 RNA or other miRNAs have been used as internal controls. While these miRNAs may be steady in several cases,



in other pathological conditions they may fluctuate; therefore, they are not reliable as an internal control.<sup>10</sup>

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conception and design of the research and obtaining financing: Abolhasani S; supervising the team: Golnoosh Mahjoob; preparing and writing of the manuscript: Yassin Ahmadi; critical revision: Huda Fatima Rajani; participated in preparation of the manuscript: Khanababaei N.

## DATA AVAILABILITY STATEMENT

The datasets collected and analyzed during the current study are available from the corresponding author on reasonable request. Furthermore, the name of repositories and reference numbers can be found in online repositories.

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