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ORIGINAL ARTICLE

The Role of Micrornas in Lipid Metabolism and their Probable Associations for Progress and Treatment of Atherosclerosis

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ABSTRACT

Introduction: Since plasma microRNAs (miRNAs) are stable in the circulatory system and are associated with a number of disorders, including coronary artery disease (CAD), the primary worldwide source of mortality and morbidity, they have been found in these conditions**.**

Objective: This study aimed to define the functions of miRNAs in lipoprotein and lipid metabolism with a focus on the conceivable participation in the onset and development of atherosclerosis.

Methods: This study was carried out at Hayatabad Medical Complex from November 2021 to March 2022. 85 patients (25 females and 60 males) diagnosed with the coronary angiography (CAG) were included because they were suspected of having CAD.

Results: The results showed that patients with CAD exhibited down-regulated expression of miRNA-199a-5`, miRNA-135a-3`, miR17-5`, and miRNA-222-3` and up-regulated expression of miRNA-144-3`, miRNA-185-5`, miRNA-133a-5`, and miRNA-222-5` in comparison to the control group. Individuals with CAD and the group that didn't take statins both had significantly higher levels of miRNA-144-3` than the control group (p=0.040 and p=0.018, respectively). Both the statin and non-statin groups significantly outperformed the control group in terms of plasma level of miRNA-133a-5` (p=0.037 and p=0.011, respectively).

Conclusion: The current study's findings revealed that CAD patients' expression levels of the miRNAs HSA miRNA-144-3`, HSA miRNA-222-5`, and HSA miRNA-133a-5` are significantly different from controls.

Keywords: microRNAs, lipid metabolism, atherosclerosis, treatment

INTRODUCTION

A low-level prolonged inflammation of an artery wall is the primary cause and mainstay of atherosclerosis, a multifactorial complicated disease [1]. A number of external stressors, for example, elevated blood pressure, oxygen radicals, or altered low-density lipoprotein (LDL) cholesterol, contribute to the dysfunction of endothelial cells that precedes the start of atherosclerosis [2]. In response, the injured endothelium starts to prompt further sticky molecules, such as vascular cell adhesion molecule 1 (VCAM-1), which promotes immune system cell attachment and infiltration [3]. A number of risk factors, including hyperlipidemia, are known to have an impact on the progression of atherosclerosis [4]. Acute hyperlipidemia promotes the progression of atherosclerosis and associated end outcomes, such as stroke or myocardial infarction as demonstrated in people having hypercholesterolemia who have these incidents during early age [5].

LDL and high-density lipoprotein (HDL) cholesterol are significant contributors to the development of hyperlipidemia and atherogenesis. Typically, proatherogenic LDL cholesterol is thought to be present, whereas antiatherogenic HDL cholesterol is present [6]. Normally, LDL-receptors clear LDL particles from the bloodstream (LDL-R). Patients with hyperlipidemia have variations in circulating lipid levels due to alterations in a number of biological pathways involved in lipid metabolism [7]. The identification of microRNAs (miRNAs, miRs), small noncoding RNA molecules known to be involved in the posttranscriptional control of gene expression, promotes the development of a more comprehensive knowledge of the atherosclerosis process [8]. Clinical research has demonstrated that severe hyperlipidemia, particularly in individuals with familial hypercholesterolemia who experience a myocardial infarction or stroke at a young age, accelerates the development of atherosclerosis and its end-point consequences, including myocardial infarction or stroke [9].

This research was conducted to characterize the functions of miRNAs involved in lipid metabolism, with a focus on the potential contribution to the onset and progress of atherosclerosis. The influence of these miRNAs as potential diagnostic and remedial methods for atherosclerosis were also studied.

MATERIALS AND METHODS

This study was conducted at Hayatabad Medical Complex, recruited 85 patients (25 women and 60 men) between November 2021 to March 2022 for this study, who had diagnosed with CAG due to the suspicion of CAD. All were interviewed regarding baseline characteristics like gender, age, bodyweight, lifestyle, and medical backgrounds in an examination room immediately after the angiographic operations. The doctor also asked the individuals about diabetes mellitus, hypertension, and other cardiovascular risk factors including hyperlipidemia. Each participant completed an informed consent form after being fully informed about the study and receiving written consent. Patients ≤18, those having renal failure, those who are severely anemia, women who are pregnant or nursing, those with malignant diseases, and those who have liver diseases are excluded from the research.

Coronary angiography: During coronary angiography (applied the Judkin method), lopromide was injected 6-8 ml at a time at each spot. Participants were labelled as CAD if at least one of their coronary arteries (CA) showed 70% stenosis. This categorization resulted in the inclusion of 51 participants with coronary artery stenosis of at least 70% and 34 subjects lacking stenosis, defining patient group and control group, accordingly. Moreover, two categories of CAD patients were created based on their use of statin therapy: a statin group (n = 24) and a non-statin group (n = 27).

Plasma collection and storage and lipid profile and CRP evaluation: All participants had peripheral blood samples (5 ml) drawn by venipuncture into tubes with EDTA. Within two hours after collection, plasma samples were kept at 80 °C prior to being spun at 2000g for 10 minutes. Triglyceride (TG) levels were determined through an enzymatic-colorimetric technique, total

cholesterol (TC), HDL cholesterol, and CRP levels were determined using an immunoturbidimetric technique. According to the Friedewald equation, the LDL and VLDL concentrations were determined.

Total RNA isolation and cDNA synthesis reaction: The whole RNA isolation technique was used in this investigation to extract miRNA. TianGen Kit was used in accordance with the manufacturer's instructions to create miRNA's cDNA and the methodology published by the Graham et al. (2021) was followed for the isolation. Using the BioMark HD System, a high throughput real-time qPCR approach was used to analyse miRNA.

Statistical analysis and plasma MiRNA expression analysis: SPSS software version 17 was used to conduct statistical analysis. Using the Shapiro-Wilk test, it was determined if the distribution of variables including triglycerides, CRP, body mass index (BMI), age, HDL, LDL, VLDL, and total cholesterol, as well as triglyceride and total cholesterol, satisfied the requirements for a normal distribution. First, the Shapiro-Wilk test was used in this research to determine the normality of the data before miRNA analysis. Because the expression of miRNAs was not evenly distributed, the Mann-Whitney test was utilised for comparison. The values are displayed mean±SD.

RESULTS

The patients' baseline and medical characteristics are outlined (Table 1). The findings indicated that there was no discernible difference between the controls and CAD patients in terms of basic characteristics. Nevertheless, there was a notable change in statin treatment between the two groups (p=0.007). By using coronary angiography, vessel stenosis was assessed (Table 1). According to coronary angiography, 12 (23.6%), 22 (43.1%) and 17 (33.3%) CAD patients had single, double and triple- or more-vessel stenosis respectively**.**

Table 1: Basic characteristics of research participants

Characteristics		Controls (n=34)	CAD (n=51)	P value
Age (years)		54.26±12.85	61.02±11.01	0.067
Gender n (%)	Female	11 (32.3)	21 (41.1)	0.366
	Male	23 (67.7)	30 (58.9)	
Hyperlipidemia n (%)	Absent	18 (52.9)	19 (37.2)	0.176
	Present	16 (47.1)	32 (62.8)	
Hypertension n (%)	Absent	20 (58.8)	22 (43.1)	0.467
	Present	14 (41.2)	29 (56.9)	
Smoking n (%)	Absent	21 (61.7)	28 (54.9)	0.392
	Present	13 (38.3)	23 (45.1)	
Alcohol n (%)	Absent	29 (85.2)	43 (84.3)	0.901
	Present	5 (14.8)	8 (15.7)	
Statin therapy n (%)	Absent	30 (88.2)	27 (52.9)	0.007
	Present	4 (11.8)	24 (47.1)	
Family History n. (%)	Absent	14 (41.2)	21 (58.9)	0.495
	Present	20 (58.8)	31 (41.1)	
No. of vessels stenosis n (%)	None	34	None	
	Single	None	12 (23.6)	
	Double	None	22 (43.1)	
	Triple or more	None	17 (33.3)	

The risk factors for CAD were analyzed in Table 2. The results showed that the odds ratio for developing CAD was 3 times higher in the absence of statin medication. This suggests that those who did not get statin medication had a 3 times higher risk than those who did. Gender is an important risk factor for CAD, and it has been shown that likelihood of producing CAD was 2.1 times greater in men (60 men) than in women (25 women) (p=0.185) when taking this into account. Furthermore, smoking and high blood pressure, both of which are significant risk factors for CAD, had odds ratios of 2.4 and 1.3, respectively. But it was discovered that family history, hyperlipidemia, and alcohol use did not increase the chance of developing CAD.

Table 2: Analysis of risk factors in patients with CAD and healthy controls

Parameters	odds ratio	95% confidence interval	P value
Gender	2.1	0.682-7.235	0.184
Smoking	2.4	0.678–7.581	0.182
Alcohol intake	0.37	0.068–1.912	0.232
Hypertension	1.3	0.442–3.446	0.692
Hyperlipidemia	0.61	0.187–2.051	0.431
Family history	0.94	0.351–2.568	0.921
Statin therapy	3.0	1.012–12.113	0.047

Patients with CAD showed greater TG, LDL- cholesterol and C-reactive protein (CRP) levels compared to controls, but lower HDL- cholesterol. The body mass index (BMI), TG, TC, LDL-C, and VLDL- cholesterol was not different significantly in both groups. Summary of patient's clinical profiles are displayed in table 3 and table 4.

Table 3: Lipid profile of healthy individuals versus CAD patients

Variables (mg/dL)	Group	n	Mean	SD	P value
TC	CAD	51	191.33	45.53	0.883
	Control	34	193.77	47.63	
LDL-C	CAD	51	123.44	35.91	0.361
	Control	34	116.90	42.57	

Table 4: BMI and CRP levels for individuals with CAD and control, as well as lipid profiles

Variables (mg/dL)	Control (n=34)	CAD (n=51)	P value
HDL-C	38 [32–52]	35 [33.50–44.25]	0.155
VLDL-C	26 [21–32]	27 [18–42]	0.617
TG	132 [96–167]	137 [91.53- 201.58]	0.738
CRP	1.92 [0.72–7.41]	3.72 [1.24–6.94]	0.113
BMI*	26 [25.4–28.43]	28.9 [25.04–31.43]	0.209

*(kg/m²)

The high-throughput qRT-PCR approach was used in the current investigation to identify the miRNA expression profile in human blood in 51 CAD patients and 34 control people. The distribution of lipid metabolism-related miRNAs that were heavily expressed in plasma was determined from the 40 (10 not expressed) miRNAs that were analyzed, were expressed in both groups. At the same time as ten (HSA miRNA-96-5`, HSA miRNA-148a-5`, HSA miRNA-758-5`, HSA miRNA-372-5`, HSAmiRNA-206, HSAmiRNA-92a-3`, HSA miRNA-27b-5`, HSA miRNA-145-5`, HSA miRNA-132-5` and HSA miRNA-221-5`) were not expressed in any of the plasma samples. Additionally, the heat map graphic of these 30 miRNAs are displayed (figure 1). In patients with CAD compared to controls, several miRNA expressions were found to be over 0.2–1.5 time's higher (figure 1). According to these findings, it has been highlighted that coronary stenosis (70%) rather than HSA mir144-3`, HSA miRNA-222-5`, and HSA miRNA-133a-5` greater expression would have especially related with the occurrence of CAD. Consequently, these miRNAs could have served as helpful early CAD indicators.

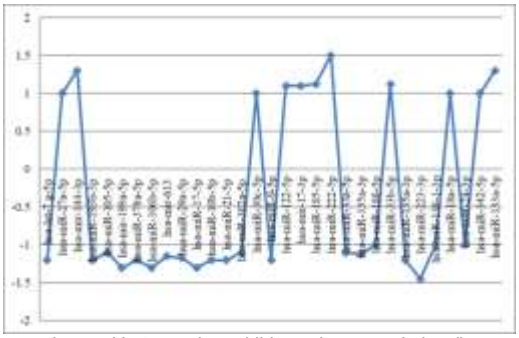

Figure 1: Patients with CAD who exhibit up-down regulation (in comparison to the control group). Information is presented as Fold Regulaton (FR). A p value of 0.05 or below was regarded as significant.

To start, we looked at whether or not miRNAs related to lipid metabolism are connected to atherosclerosis, the primary cause of which is hyperlipidemia. A related question is whether statins, the only treatment for CAD, have an impact on the expression of miRNAs involved in lipid metabolism. We divided CAD patients into two groups, one for those who did not take statins and the other for those who did, based on this assumption (Table 5). The findings demonstrated that HSA miRNA-144-3` has been substantially expressed in the non-statin (FR = 1.33, p = 0.018), but HSA miRNA-222-5` was considerably expressed in the statin (FR = 1.47, p = 0.032).

The impacts of coronary stenosis severity on miRNA expression level were also identified by our research. Patients in Group (1) have at least one CA that is 70% or more stenosed; patients in Group (2) have two CA that are 70% or more stenosed; patients in Group (3) have three or more CA that are 70% or more stenosed; and patients in Group (4), who don't have any CA stenosis (<70%), are referred to as controls. Between groups 1-3, we compared results. Groups 1 and 3 both had considerably higher levels of HSA miRNA-27a-5` and group 3 had significantly higher levels of HSA mir144-3` (p=0.001, 0.040, and 0.012, respectively) in comparison to the control group. While HSA miRNA-222-5` was considerably upregulated in only group 2 (p=0.014), HSA miRNA-122-5` was significantly upregulated in group 1 (p=0.012). In all three groups, HSA miRNA-17-3` was up-regulated (p=0.048, p=0.001, and p=0.002). In addition, only group 2 showed u-regulation of HSA miRNA-133a-5` (p=0.003) (Table 6).

Table 5: Comparing CAD with the controls by statin treatment.

Gene ID	Non-statin group		Statin group	
	FR	P value	FR	P value
HSA let 7 g-5`	1.143	0.311	−1.245	0.526
HSA miRNA-27a-5`	1.057	0.246	1.001	0.001
HSA miRNA-144-3`	1.33	0.018	1.002	0.001
HSA miRNA-193b-3`	−1.108	0.295	−1.108	0.336
HSA miRNA-105-5`	−1.031	0.395	−1.031	0.436
HSA miRNA-199a-5` −	−1.108	0.295	−1.108	0.338
HSA miRNA-378a-5`	−1.052	0.457	−1.092	0.342
HSA miRNA-613	−1.041	0.397	−1.044	0.438
HSA miRNA-106b-5`	1.466	0.593	−1.471	0.322
HSA miRNA-29a-5`	−1.022	0.392	−1.023	0.438
HSA miRNA-21-5`	1.309	0.607	1.248	0.779
HSA miRNA-10b-5`	1.009	0.968	−1.075	0.268
HSA miRNA-17-5`	1.116	0.521	−1.557	0.316
HSA miRNA-302a-5`	−1.002	0.841	1.033	0.535
HSA miRNA-30c-5`	1.162	0.402	1.211	0.358
HSA miRNA-122-5`	1.108	0.173	1.287	0.715
HSA miRNA-17-3`	1.072	0.247	1.000	0.000
HSA miRNA-9-5`	1.108	0.173	1.282	0.717
HSA miRNA-185-5`	−1.002	0.843	1.037	0.535
HSA miRNA-222-5`	1.47	0.055	1.47	0.032
HSA miRNA-33a-5`	−1.031	0.392	−1.034	0.433
HSA miRNA-135a-3`	−1.108	0.293	−1.102	0.336
HSA miRNA-188-5`	1.465	0.592	−1.470	0.321
HSA miRNA-223-3`	−1.53	0.273	−1.19	0.619
HSA miRNA-33b-5`	1.116	0.521	−1.558	0.318
HSA miRNA-138-1-3`	−1.107	0.294	−1.106	0.336
HSA miRNA-18a-5`	1.164	0.405	1.216	0.365
HSA miRNA-133a-5`	1.290	0.037	1.34	0.011
HSA miRNA-24-3`	−1.108	0.293	−1.102	0.331
HSA miRNA-342-5`	1.164	0.406	1.217	0.358

Table 6: Regulation of plasma miRNAs in individuals with CAD greater than and equal to 70% compared to those with CAD of less than 70% or normal.

Gene ID	Number of vessel stenosis					
	Single		Double		Triple or more	
	FR	p value	FR	p value	FR	p value
HSA let 7 g-5`	−1.002	0.843	1.164	0.405	1.216	0.365
HSA miRNA-27a-5`	1.000	0.000	1.071	0.154	1.000	0.000
HSA miRNA-144-3`	1.000	0.001	1.190	0.040	1.277	0.012
HSA miRNA-193b-3`	−1.044	0.438	1.108	0.173	1.282	0.717
HSA miRNA-105-5`	−1.471	0.322	−1.002	0.843	1.037	0.535
HSA miRNA-199a-5`	−1.107	0.294	1.47	0.055	1.41	0.032
HSA miRNA-378a-5`	1.164	0.405	−1.031	0.392	−1.034	0.433
HSA miRNA-613	−1.108	0.295	−1.108	0.338	−1.041	0.397
HSA miRNA-106b-5`	1.47	0.032	−1.108	0.295	−1.108	0.338
HSA miRNA-29a-5`	−1.044	0.438	−1.052	0.457	−1.092	0.342
HSA miRNA-21-5`	−1.471	0.322	−1.041	0.397	−1.044	0.438
HSA miRNA-10b-5`	−1.023	0.438	1.466	0.593	−1.471	0.322
HSA miRNA-17-5`	1.24	0.779	−1.107	0.294	−1.106	0.336
HSA miRNA-302a-5`	1.41	0.032	1.164	0.405	1.216	0.365
HSA miRNA-30c-5`	1.47	0.032	1.466	0.593	−1.471	0.322
HSA miRNA-122-5`	1.618	0.012	1.102	0.322	−1.531	0.449
HSA miRNA-17-3`	1.171	0.048	1.000	0.001	1.000	0.002
HSA miRNA-9-5`	−1.471	0.322	−1.041	0.397	−1.04	0.438
HSA miRNA-185-5`	−1.023	0.438	1.466	0.593	−1.471	0.322
HSA miRNA-222-5`	1.438	0.125	1.809	0.014	1.043	0.324
HSA miRNA-33a-5`	−1.022	0.392	−1.023	0.438	−1.044	0.438
HSA miRNA-135a-3`	−1.108	0.295	−1.108	0.338	−1.471	0.322
HSA miRNA-188-5`	−1.052	0.457	−1.092	0.342	−1.023	0.438
HSA miRNA-223-3`	−1.041	0.397	−1.044	0.438	1.248	0.779
HSA miRNA-33b-5`	1.466	0.593	−1.471	0.322	0.593	−1.471
HSA miRNA-138-1-3`	−1.022	0.392	−1.023	0.438	0.014	1.043
HSA miRNA-18a-5`	1.309	0.607	1.248	0.779	0.438	−1.044
HSA miRNA-133a-5`	1.147	0.151	1.486	0.003	1.197	0.067
HSA miRNA-24-3`	−1.023	0.438	1.466	0.593	−1.471	0.322
HSA miRNA-342-5`	1.438	0.125	1.809	0.014	1.043	0.324

DISCUSSION

Different metabolic disorders, including atherosclerosis, are brought on by lipid metabolism disorders. Recent research has demonstrated that miRNAs are crucial for controlling lipid metabolism, including the synthesis of triglycerides and the absorption of cholesterol and fatty acids [10]. To ascertain the potential consequences of atherosclerosis development, we looked at the regulation levels of miRNAs that are essential for lipid metabolism in the present research. According to the current study, plasma taken from atherosclerosis patients and healthy

controls, we examined the expression levels of 40 miRNAs. To identify which miRNAs had varying expression level in patient`s group, we first compared the CAD patients to the control group in our study. The findings demonstrated that as compared to the control group, patients with CAD had up-regulated expression of miRNA-144-3`, miRNA-185-5`, miRNA-133a-5`, and miRNA-222-5` and down-regulated expression of miRNA-199a-5`, miRNA-135a-3`, miR17-5`, and miRNA-222-3`. On the basis of these findings, we hypothesised that the miRNAs would play significant roles in controlling a number of genes associated with lipid metabolism genes implicated in the development of atherosclerosis.

According to Ramirez et al., (2013) miR144 expression in macrophages, hepatocytes, and endothelial cells reduced cellular cholesterol efflux and inhibited ABCA1 expression, which in turn controlled cholesterol homeostasis [11]. Similar to this, Yin et al., (2016) found that the plasma of CAD patients had increased miRNA-144 levels [12]. Our findings demonstrated that, compared to the control group, patients with CAD and the non-statin usage group both had substantially higher levels of miRNA-144-3` ($p=0.040$ and $p=0.018$, respectively). Based on the findings, we understand that miRNA-144-3` expression levels may influence the course and severity of atherosclerosis by accumulating lipids in macrophages, and it has been hypothesised that miRNA-144 may one day function as an efficient therapeutic strategy or drug for the treatment of atherosclerosis and as a valuable biomarker.

In contrast to healthy control groups, it was found that miRNA-133a was substantially differently expressed in a variety of circulatory disorders [13]. According to Fichtlscherer et al. (2010), considerably higher expression of miRNA-133a were present in the individuals having CAD in the cardiac muscle ($p=0.017$) [14]. Our research showed that both the non-statin use group and the statin use group had substantially higher miRNA-133a-5` plasma levels than the control group ($p=0.037$ and $p=0.011$, respectively). Comparing the double vessel stenosis group to the control, miRNA-133a-5` levels were similarly greater ($p=0.003$). The correlation between elevated levels of miRNA-133a and the development of CA disease is not entirely assumed, despite the fact that our results were comparable to those of these previous investigations. With the information at hand, we predicted that elevated levels of miRNA-133a in these individuals may have served as a prognostic indicator and a therapeutic target, but further research is still required.

According to Vickers et al., (2014), miRNA-223 controls the three main mechanisms that control the levels of intracellular and systemic cholesterol: cholesterol production, uptake, and excretion [15]. When compared to those with atypical chest pain, patients with ST-segment elevation myocardial infarction have a little lower amount of miRNA-223 in their plasma, it has been shown in another study that miRNA-223 is not a robust biomarker for the condition [16].Similar to this, our findings showed that the expression level of miR223 was lower in the statin group than it was in the non-statin group (FR = 1.53 and 1.19, respectively). Furthermore, in our study, the CAD group's miRNA-222-5` levels were considerably higher than those of the control group.

CONCLUSION

In summary, the results of the current investigation showed that the expression levels of the miRNAs HSA miRNA-144-3`, HSA miRNA-222-5`, and HSA miRNA-133a-5` are substantially different in CAD patients compared to controls. When we compare the groups based on vascular stenosis and statin drug usage, these miRNAs have a variety of effects as well. We proposed that these miRNAs may have served as helpful biomarkers for the early identification of CAD patients. The identification of medications and therapies for the control of lipid metabolism in the development of atherosclerosis will be aided by understanding the function of miRNAs in the development of atherosclerosis, based on the findings of our study.

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