

Expression of miRNA-27a in the serum of patients with non-ST elevation acute coronary syndrome who underwent percutaneous coronary intervention

Draganova A. S.^{1,2}, Polyakova E. A.^{1,2}, Kolodina D. A.¹, Mikheeva K. Yu.¹, Belyaeva O. D.^{1,2}, Zaraysky M. I.¹, Berkovich O. A.^{1,2}, Shlyakhto E. V.^{1,2}

Coronary artery disease (CAD) is a multifactorial disorder. Previously have been identified genes whose polymorphic variants are associated with an increased risk of CAD. Genetic control of the development of CAD at the post-transcriptional level is carried out using step-wise and multicomponent regulation of gene expression with the participation of specific molecules called micro-ribonucleic acids (miRNAs). Currently, many authors consider these molecules, in particular miRNA-27a, as potential sensitive diagnostic markers for acute coronary syndrome (ACS).

Aim. To assess the level of miRNA-27a expression in the serum of patients underwent percutaneous coronary intervention (PCI) after non-ST elevation ACS.

Material and methods. Forty patients with non-ST elevation ACS who underwent coronary artery stenting were examined. The comparison groups consisted of 80 patients with a stable CAD who underwent coronary artery bypass surgery, and 20 patients without clinical signs of CAD operated due to valvular disorders without atherosclerotic lesions. All patients underwent coronary angiography. The expression level of miRNA-27a was determined in serum by real-time polymerase chain reaction.

Results. In patients with non-ST elevation ACS, who underwent PCI, the expression level of miRNA-27a in serum was higher than in patients without atherosclerotic lesions ($6,99 \pm 1,69$ and $3,05 \pm 0,89$, respectively; $p0,05$).

Conclusion. High levels of miRNA-27a expression can be considered as a marker of coronary lesion severity in patients with CAD, but not as a marker for ACS.

Key words: micro-RNA, coronary artery disease, acute coronary syndrome.

Conflicts of Interest: nothing to declare.

¹First Pavlov State Medical University of St. Petersburg, St. Petersburg; ²Almazov National Medical Research Centre, St. Petersburg, Russia.

Draganova A. S.* ORCID: 0000-0002-9541-0947, Polyakova E. A. ORCID: 0000-0002-3231-6152, Kolodina D. A. ORCID: 0000-0003-2889-0706, Mikheeva K. Yu. ORCID: 0000-0001-9450-7758, Belyaeva O. D. ORCID: 0000-0002-5349-2227, Zaraysky M. I. ORCID: 0000-0002-7605-4369, Berkovich O. A. ORCID: 0000-0002-5358-5968, Shlyakhto E. V. ORCID: 0000-0003-2929-0980.

*Corresponding author: anchous0_0@mail.ru

Received: 13.11.2018

Revision Received: 24.01.2019

Accepted: 31.01.2019



For citation: Draganova A.S., Polyakova E.A., Kolodina D.A., Mikheeva K.Y., Belyaeva O.D., Zaraysky M.I., Berkovich O.A., Shlyakhto E.V. Expression of miRNA-27a in the serum of patients with non-ST elevation acute coronary syndrome who underwent percutaneous coronary intervention. *Russian Journal of Cardiology*. 2019;(2):70-75. (In Russ.) doi:10.15829/1560-4071-2019-2-70-75

Cardiovascular diseases (CVD) and foremost coronary heart disease (CAD) are one of the most important medical and social problems due to its high share in the patterns of morbidity, disability and mortality. Despite the progress in primary prevention and pharmacotherapy, as well as the high efficiency of surgical methods for the CAD treatment, in particular percutaneous coronary interventions (PCI), today CAD is a leading cause of death worldwide. The annual incidence of acute coronary syndrome (ACS) in Europe remains high and varies from 1:80 to 1:170 people per year according to various registers. In this regard, there is a need to search for new molecular genetic markers of CAD (including ACS) severity.

The number of publications on this problem is constantly increasing. However, little is still known about the post-transcriptional regulation of the expression of CAD candidate genes. Post-transcriptional gene regulation is a change in the RNA structure until the start of gene translation. One of the main processes of post-transcriptional regulation is RNA interference. RNA interference is a process directed by special regulatory molecules — non-coding small interfering RNA — microRNA (miRNA) [1, 2].

It is known that miRNAs plays an important role in the CVD development by participating in various biological processes, such as endothelial dysfunction, cell adhesion, plaque formation and rupture, angiogenesis [3, 4], proliferation, metabolism, and apoptosis [5].

In recent years, special attention has been paid to the study of miRNA circulating in the blood, which can be used as markers for minimally invasive diagnosis of CVD, including some forms of CAD. So, it is known that a number of miRNA can be considered as ACS markers [6-8].

There are few contradictory studies about miRNA-27a, which associated with ACS [9-11].

In this regard, the aim of this study was to evaluate the level of miRNA-27a expression in blood serum in patients with non-ST-segment elevation ACS after PCI.

Material and methods

The study was approved by the Local Ethics Committee of the First Pavlov State Medical University of St. Petersburg; all patients signed an informed consent.

The study included 40 people (28 men and 12 women) with CAD after PCI due to ACS. All patients underwent coronary angiography to determine the

nature of coronary artery injury and management tactics. Patients were divided into 2 groups. The first group consisted of 19 patients (48%) with diagnosed one- or two-vessel CAD, the second group — 21 patients (51%) with multivessel CAD (3 arteries and more). The comparison group consisted of 20 (10 men and 10 women) subjects without CAD, according to coronary angiography.

Also, as an additional comparison group, 80 patients with a stable CAD (57 men and 23 women) and clinical picture of angina pectoris, which later underwent planned coronary artery bypass grafting (CABG), were examined.

Exclusion criteria were previous CABG, thyroid disease, secondary obesity and hypertension, oncology, acute kidney injury and chronic kidney disease, systemic connective tissue diseases, infective endocarditis, hypo/hyperthyroidism, organic brain diseases, alcoholism, drug addiction.

All patients with ACS received therapy in accordance with the guidelines for the management of ACS patients. The following data were collected from all patients: smoking history, family history of CVD, presence of overweight/obesity. A general examination was performed; height, body weight, waist circumference (WC) were measured and body mass index (BMI) was calculated. All biochemical parameters were determined on an automatic biochemistry analyzer (COBAS INTEGRA 400/700/800) with standard Roche kit (Germany). Quantitation of venous plasma glucose by hexokinase method was performed. Serum lipids (total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C)) was analyzed by enzymatic method.

Blood sampling was carried out no longer than 48 hours after the ACS onset and before CABG in patients with stable angina pectoris. Molecular genetic tests were performed at the Department of Clinical Laboratory Diagnostics of First Pavlov State Medical University of St. Petersburg. Blood serum was used in miRNA isolation. miRNA was isolated using the miRNeasy Mini Kit (QIAGEN, USA). The concentration of the miRNA aqueous solution was determined on a Nanodrop 1000 spectrophotometer (Thermo Scientific, USA). Reverse transcription was performed using a TaqMan miRNA reverse transcription kit. Real-time polymerase chain reaction was carried out using a miRNA-27a expression kit manufactured by Applied Biosystems (USA) TaqMan® Gene Expression Assays.

Statistical processing was carried out using the parametric and nonparametric methods. The level

of miRNA-27a had a normal distribution. Logarithm of miRNA determination results were taken to stabilize the dispersion and symmetrize the distribution law. The data are presented as an estimate of the arithmetic mean (M) and error of the mean (m). To assess the intergroup differences, the Wilcoxon-Mann-Whitney U test was used. When comparing frequency values, the Pearson's chi-squared test and Fisher's exact test were used. A multivariate regression analysis was performed to establish the association of miRNAs with a combination of various indicators potentially affecting it. The logarithmic miRNA was used as a dependent variable. Anamnestic, clinical, laboratory and functional parameters were used as independent explicative variables. For the diagnosis of multicollinearity, the VIF (variance inflation factor) was calculated and considered significant if it was less than two. Statistical processing was performed using SPSS 20.0 for Windows. The critical significance level of the null hypothesis (the absence of differences and influences) was taken equal to $<0,05$.

Results

Patients with non-ST segment elevation ACS after PCI of groups 1A (one- or two-vessel CAD) and 1B (multivessel CAD), patients without coronary atherosclerotic lesions (comparison group 1) and patients with stable CAD (comparison groups 2A and 2B) were comparable in age (Table 1).

Among the modifiable CVD risk factors, smoking was the most common in the group of patients with ACS. Among patients with ACS, 62,5% of patients smoked, which is significantly more than among patients without CAD ($p<0,05$). The level of miRNA-27a expression in blood serum in smokers and non-smokers with ACS did not differ ($p>0,05$).

It was shown that 12,5% of patients with ACS had a positive family history of CVD; the level of miRNA-27a expression in blood serum in patients with/without this risk factor also did not differ ($p>0,05$).

Anthropometric parameters were analyzed in patients with ACS and comparison groups. Abdominal obesity (AO) was verified according to the criteria of the International Diabetes Federation (IDF, 2005). According to these criteria, AO were in 46% of men ($n=13$) and 42% of women ($n=5$). The average WC values in patients with ACS of 1A and 1B groups did not differ, but in men with ACS and stable CAD, WC was greater

than in men without CAD ($p<0,01$) (Table 1). In women, such differences were not detected ($p>0,05$) (Table 1).

When assessing the level of miRNA-27a depending on AO presence/absence in men and women, WC was recalculated from a quantitative to qualitative character taking into account different standards for men and women. There were no significant differences in the level of miRNA-27a in patients with and without AO ($p>0,05$).

When assessing miRNA-27a level in patients with ACS in groups with normal body weight, overweight and class I-III obesity, there were no significant differences in miRNA-27a level ($p>0,05$).

When assessing the serum lipid, it was found that TC and LDL-C levels in patients with ACS and patients with a stable CAD did not differ, but were lower than in patients without CAD (Table 1). Probably, these differences are due to the regular use of HMG-CoA reductase inhibitors in patients with CAD.

The expression level of miRNA-27a in men and women of groups 1A and 1B did not differ ($p>0,05$).

20% of patients with ACS had type 2 diabetes (T2D). It was found that the expression level of miRNA-27a in patients with CAD and T2D and without T2D did not significantly differ ($p>0,05$).

It was found that in patients with CAD after non-ST segment elevation ACS, and in patients with a stable CAD, the level of miRNA-27a expression in blood serum was higher than in patients without CAD ($6,99\pm1,69$ RU, $7,82\pm1,79$ RU and $3,05\pm0,89$ RU, respectively; $p<0,05$). Moreover, both patients with ACS and patients with stable CAD and multivessel coronary lesions had a higher level of miRNA-27a serum expression than patients with one- or two-vessel CAD ($p<0,01$) (Table 1).

At the same time, the level of miRNA-27a serum expression in patients with non-ST segment elevation ACS (groups 1A and 1B) and in patients with stable CAD (groups 2A and 2B) did not differ ($6,99\pm1,69$ RU and $8,57\pm3,90$ RU, respectively; $p>0,05$).

A comparative analysis of miRNA-27a serum expression in patients with ACS revealed the following: in patients with hemodynamically significant stenoses of the diagonal branch of the left coronary artery and the posterolateral branch of the right coronary artery than patients with coronary artery stenosis less than 70%, higher levels of miRNA-27a expression ($5,2\pm1,44$ RU and

Table 1

General characteristics and serum miRNA-27a expression level in participants

Parameter	Group 1A Patients with non-ST segment elevation ACS (one- or two- vessel lesion) n=19 (48%)	Group 1B Patients with non-ST segment elevation ACS (multivessel lesion) n=21 (52%)	Comparison Group 1 Patients without CAD n=20	Comparison Group 2A Patients with stable CAD (one- or two- vessel lesion) n=29 (36%)	Comparison Group 2B Patients with stable CAD (multivessel lesion) n=51 (64%)	p
miRNA-27a, RU	5,87±2,64	8,00±2,19	3,05±0,89	4,82±1,82	11,41±4,45	$p_{1-1A;1-1B;1-2A;1-2B}<0,05$ $p_{1A-1B}<0,01$ $p_{2A-2B}<0,01$
Age, years	63,05±2,66	64,86±1,80	59,13±3,47	61,50±2,30	62,50±2,40	NS
Waist circumference in men, cm	105,53±3,57	104,58±4,44	95,32±4,37	99,80±3,90	100,80±4,20	$p_{1-1A;1-1B;1-2A;1-2B}<0,01$
Waist circumference in women, cm	89,60±5,77	88,30±4,81	81,86±5,84	88,50±4,20	89,20±4,40	NS
Body mass index, kg/m ²	28,02±1,17	28,70±1,27	25,44±1,08	28,40±1,90	28,30±1,80	NS
Total cholesterol, mmol/l	4,37±0,15	4,23±0,13	5,18±0,19	4,70±0,20	4,30±0,20	$p_{1-1A;1-1B;1-2A;1-2B}<0,001$
Low density lipoprotein cholesterol, mmol/l	1,51±0,15	1,18±0,12	3,08±0,14	2,40±0,30	2,10±0,20	$p_{1-1A;1-1B;1-2A;1-2B}<0,001$
High density lipoprotein cholesterol, mmol/l	1,27±0,09	1,28±0,06	1,26±0,14	1,10±0,10	1,10±0,10	NS
Triglycerides, mmol/l	1,78±0,16	1,65±0,13	1,20±0,16	1,80±0,20	1,90±0,10	$p_{1-1A;1-1B;1-2A;1-2B}<0,05$
Blood glucose, mmol/l	5,89±0,18	6,47±0,32	5,41±0,34	5,80±0,20	5,90±0,20	$p_{1-1A;1-1B;1-2A;1-2B}<0,001$

Abbreviation: NS — not significant.

9,33±3,10 RU, respectively; $p<0,04$; 1,58±0,51 RU and 7,76±1,90 RU, respectively; $p<0,05$) were detected.

When conducting a correlation analysis in ACS group, a positive correlation was found between miRNA-27a expression level and total number of implanted stents ($r=0,327$, $p=0,04$). Moreover, when performing multivariate regression analysis, the factor potentially affecting the serum miRNA-27a expression level was the number of implanted stents ($p=0,001$, VIF=1,03, $r=0,42$).

Discussion

In recent decades, complex approach to the CVD diagnosis and treatment is most relevant; CAD as a multifactorial disease is no exception. According to modern concepts, the most important factors in the CAD development and progression are not only well-known cellular and biochemical markers, but also genetic and epigenetic factors. A special role among epigenetic factors is played by miRNA that regulate post-transcriptional gene expression. To date, more than 1800

human miRNAs are known and this list is constantly rising.

To date, a number of miRNAs are considered as new diagnostic and prognostic markers in patients with various CVDs. The use of such markers may become relevant and appropriate in clinical practice, taking into account the its relative simplicity and accessibility [12].

Despite a large number of studies about the miRNA-27a role in coronary artery atherosclerosis and stable CAD, there are few studies that evaluated miRNA-27a level in ACS. In the study by Shvangiradze TA, et al. (2016) [9], where only patients with a stable CAD and T2D were included, it was found that the miRNA-27a expression level was higher in the T2D and CAD group than in T2D patients without CAD. In our study, it was found that serum miRNA-27a expression level was higher in patients with a stable CAD and ACS than in those without atherosclerotic coronary lesions, which is consistent with the results of the study by Alvarez M, et al. [12] and other recent studies by Aranda JF, et al. [13] and Chen W-J, et al. [14].

Moreover, in our study, it was found that in multivessel CAD, the miRNA-27a expression level is higher than in one- and two-vessel lesions. In a study by Devaux Y, et al. [10] miRNA-27a prognostic significance in patients after ACS was evaluated. It was shown that an increased miRNA-27a expression level was associated with adverse clinical outcomes after myocardial infarction. That, according to the authors, may be due to a more severe coronary lesion in this category of patients. However, there are no publications on miRNA-27a

expression level in multivessel and one- and two-vessel lesions.

In our study, no evidence was obtained indicating that miRNA-27a may be a marker of ACS. This is consistent with a study by Kukreja R, et al. [11].

During the correlation analysis, we revealed a positive correlation between the miRNA-27a level and total number of implanted stents. Moreover, multivariate regression analysis showed that the total number of implanted stents may be a factor potentially affecting miRNA-27a serum level. This suggests that miRNA-27a can take part in the neovascularization and endothelial dysfunction, including those associated with stenting. This is an important mechanism that is also being discussed when studying the miRNA-27a role in CAD patients after PCI. So, Veliceasa D, et al. [15], studied the miRNA-27b role, related to miRNA-27a, in an experimental ischemia model in mice and demonstrated that in critical ischemia miRNA-27b increase vascularization, decrease fibrosis, activate tissue revascularization and perfusion. The authors of this study suggest that these effects are possibly due to a decrease in the expression of delta-like protein 4, interleukin-10 and receptors activated by peroxisome proliferators.

Conclusion

Based on the data obtained, it can be assumed that higher miRNA-27a expression level is associated with extended, clinically significant coronary atherosclerosis in patients with non-ST segment elevation ACS and in patients with stable CAD, but is not an ACS marker.

Conflicts of Interest: nothing to declare.

References

1. Sárközy M, Kahán Z, Csont T, et al. A myriad of roles of miR-25 in health and disease. *Oncotarget*. 2018; 9:21580-612. doi:10.18632/oncotarget.24662.
2. Macgregor-Das A, Das S. A microRNA's Journey to the Center of the Mitochondria. *American journal of physiology*. 2018;315(2):H206-H215. doi:10.1152/ajpheart.00714.2017.
3. Janaszak-Jasiecka A, Siekierzycka A, Bartoszevska S, et al. eNOS expression and NO release during hypoxia is inhibited by miR-200b in human endothelial cells. *Angiogenesis*. 2018;21(4):711-24. doi:10.1007/s10456-018-9620-y.
4. Lino M, Simões S, Vilaça A, et al. Modulation of Angiogenic Activity by Light-Activatable miRNA-Loaded Nanocarriers. *ACS Nano*. 2018. doi:10.1021/acsnano.7b07538. [Epub ahead of print];
5. Tsoporis J, Fazio A, Rizos IK, et al. Increased right atrial appendage apoptosis is associated with differential regulation of candidate MicroRNAs 1 and 133A in patients who developed atrial fibrillation after cardiac surgery. *J Mol Cell Cardiol*. 2018. doi:10.1016/j.yjmcc.2018.06.005. [Epub ahead of print];
6. Gacoń J, Kabłak-Ziembicka A, Stępień E, et al. Decision-making microRNAs (miR-124, -133a/b, -34a and -134) in patients with occluded target vessel in acute coronary syndrome. *Kardiologia Polska*. 2016;74(3):280-8. doi:10.5603/KP.a2015.0174.
7. Gacoń J, Badacz R, Stępień E, et al. Diagnostic and prognostic micro-RNAs in ischaemic stroke due to carotid artery stenosis and in acute coronary syndrome: a four-year prospective study. *Kardiologia Polska*. 2018;76(2):362-9. doi:10.5603/KP.a2017.0243.
8. Li S, Fan Q, He S, et al. MicroRNA-21 negatively regulates Treg cells through a TGF- β 1/Smad-independent pathway in patients with coronary heart disease. *Cell Physiol Biochem*. 2015;37(3):866-78. doi:10.1159/000430214.
9. Shvangiradze TA, Bondarenko IZ, Troshina EA, et al. Profile of microRNAs associated with coronary heart disease in patients with type 2 diabetes. *Obesity and metabolism*. 2016;13(4):34-8. (In Russ.) doi:10.14341/OMET2016434-38.
10. Devaux Y, Vausort M, McCann GP, et al. A panel of 4 microRNAs facilitates the prediction of left ventricular contractility after acute myocardial infarction. *PLoS ONE*. 2013;8(8):e70644. doi:10.1371/journal.pone.0070644.e70644.
11. Kukreja R, Yin C, Salloum FN, et al. MicroRNAs: New Players in Cardiac Injury and Protection. *Mol Pharmacol*. 2011 Oct;80(4):558-64. doi:10.1124/mol.111.073528.
12. Alvarez M, Khosroheidari M, Eddy E, et al. MicroRNA-27a decreases the level and efficiency of the LDL receptor and contributes to the dysregulation of cholesterol homeostasis. *Atherosclerosis*. 2015;242(2):595-604. doi:10.1016/j.atherosclerosis.2015.08.023.
13. Aranda J, Madrigal-Matute J, Rotllan N, et al. MicroRNA modulation of lipid metabolism and oxidative stress in cardiometabolic diseases. *Free Radic Biol Med*. 2013;64:31-9. doi:10.1016/j.freeradbiomed.2013.07.014.
14. Chen W, Yin K, Zhao GJ, et al. The magic and mystery of MicroRNA-27 in atherosclerosis. *Atherosclerosis*. 2012;222(2):314-23. doi:10.1016/j.atherosclerosis.2012.01.020.
15. Veliceasa D, Biyashev D, Qin G, et al. Therapeutic manipulation of angiogenesis with miR-27b. *Vasc Cell*. 2015;7:6. doi:10.1186/s13221-015-0031-1.