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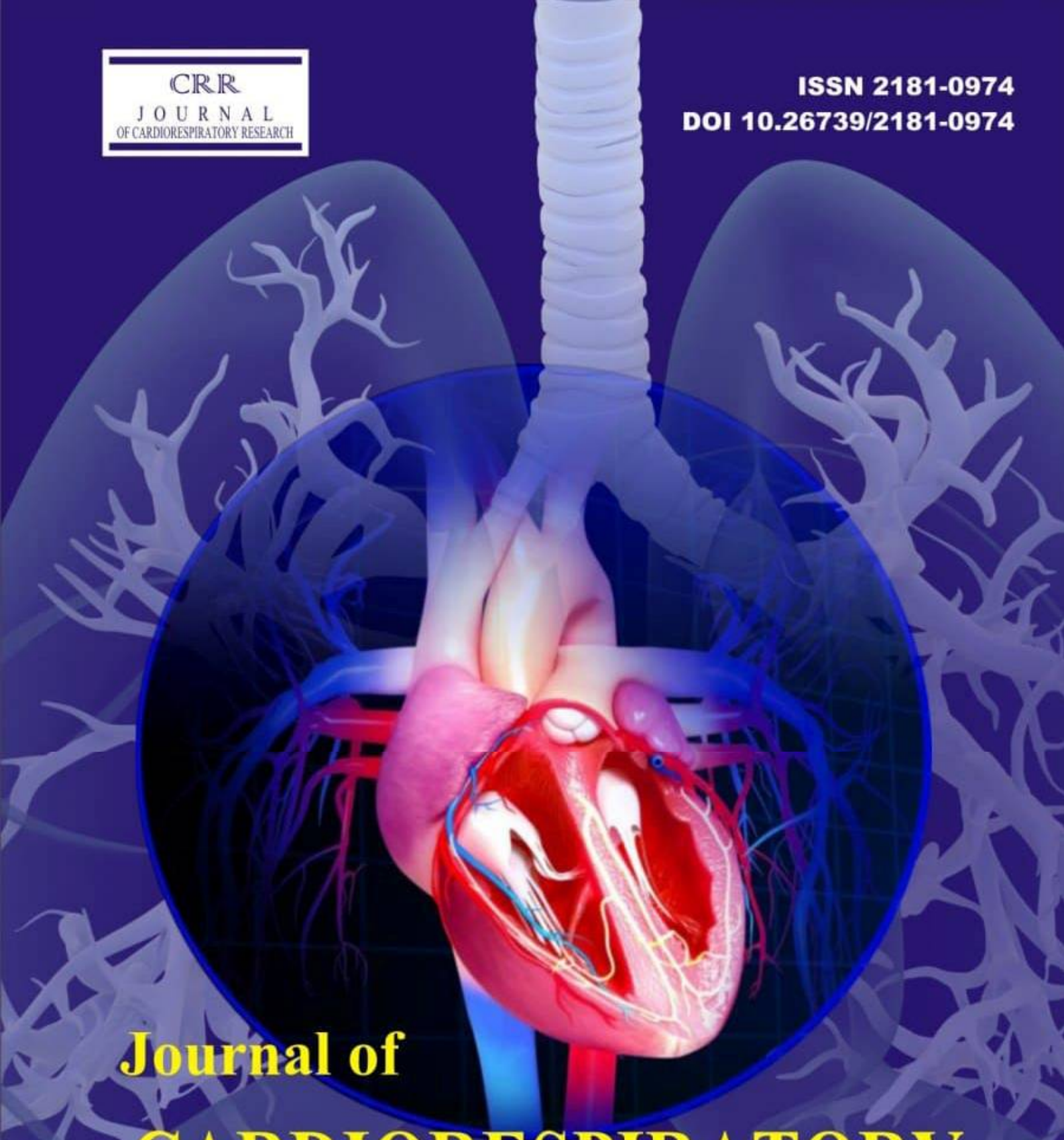

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JOURNAL OF CARDIORESPIRATORY RESEARCH
ЖУРНАЛ КАРДИОРЕСПИРАТОРНЫХ ИССЛЕДОВАНИЙ

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ФАКТОРЫ СЕРДЕЧНО-СОСУДИСТОГО РИСКА И УРОВЕНЬ ЭКСПРЕССИИ МИКРОРНК У БОЛЬНЫХ ИНФАРКТОМ МИОКАРДА

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АННОТАЦИЯ

Сердечно-сосудистые факторы риска способствуют развитию сердечно-сосудистых заболеваний, и их присутствие может влиять на уровень экспрессии микроРНК у пациентов с инфарктом миокарда с подъемом сегмента ST. До сих пор было опубликовано лишь несколько сообщений, описывающих взаимосвязь между экспрессией микроРНК и наличием сердечно-сосудистых факторов риска.

Цель

Оценка экспрессии микроРНК у пациентов с инфарктом миокарда с подъемом сегмента ST и наличием сердечно-сосудистых факторов риска.

Материалы и методы

Обследовано 17 больных инфарктом миокарда с подъемом сегмента ST. Экспрессию микроРНК (миР-1, миР-208а и миР-423-5р) определяли по модели Пфаффла, а затем сравнивали между двумя группами: с факторами риска сердечно-сосудистых заболеваний и без них.

Полученные результаты

Более высокие уровни экспрессии микроРНК обнаружены у курильщиков. Более низкие уровни экспрессии miR-1, miR-208a и miR-423-5p были обнаружены у пациентов с повышенным уровнем липидов или у пациентов, принимающих гиполипемические препараты.

Вывод

Экспрессия микроРНК у пациентов с инфарктом миокарда с подъемом сегмента ST зависит от наличия некоторых сердечно-сосудистых факторов риска.

Ключевые слова: сердечно-сосудистые факторы риска, микроРНК, инфаркт миокарда.

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CARDIOVASCULAR RISK FACTORS AND EXPRESSION LEVEL OF MICRORNA IN PATIENTS WITH MYOCARDIAL INFARCTION

ANNOTATION

Cardiovascular risk factors contribute to the development of cardiovascular disease and their presence may affect the level of microRNA expression in patients with ST-elevation myocardial infarction. So far, only a few reports have been published describing the relationship between microRNA expression and the presence of cardiovascular risk factors.

Aim

Evaluation of microRNA expression in patients with ST- elevation myocardial infarction with present cardiovascular risk factors.

Materials & Methods

Seventeen patients with ST-elevation myocardial infarction were examined. MicroRNA expression (miR-1, miR-208a i miR-423-5p) was determined according to the Pfaffla model and then compared between two groups: with and without cardiovascular risk factors.

Results

Higher expression levels of microRNAs were found in smokers. Lower levels of miR-1, miR-208a and miR-423-5p expression were found in patients with increased lipid levels or these using hypolipemic drugs.

Conclusion

MicroRNA expression in patients with ST-elevation myocardial infarction depends on the presence of some cardiovascular risk factors.

Keywords: Cardiovascular risk factors, microRNA, myocardial infarction.

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MIOKARD INFARKTI BO'LGAN BEMORLARDA YURAK-QON TOMIR XAVF OMILLARI VA MIKRORNKNING IFODA DARAJASI

ANNOTATSIYA

Yurak-qon tomir xavf omillari yurak-qon tomir kasalliklarining rivojlanishiga yordam beradi va ularning mavjudligi ST ko`tarilishi bilan miokard infarkti bo'lgan bemorlarda mikroRNK ifodasi darajasiga ta'sir qilishi mumkin. Hozirgacha mikroRNK ifodasi va yurak-qon tomir xavf omillarining mavjudligi o'rtasidagi munosabatni tavsiflovchi bir nechta hisobotlar nashr etilgan.

Maqsad

Hozirgi yurak-qon tomir xavf omillari bilan ST ko'tarilishi bilan miokard infarkti bo'lgan bemorlarda mikroRNK ifodasini baholash.

Materiallar va usullar

ST darajasi yuqori bo'lgan miokard infarkti bo'lgan 17 nafar bemor tekshirildi. MicroRNK ifodasi (miR-1, miR-208a i miR-423-5p) Pfaffla modeli bo'yicha aniqlandi va keyin ikki guruh o'rtasida taqqoslandi: yurak-qon tomir xavf omillari bilan va ularsiz.

Natijalar

Sigaret chekuvchilarda mikroRNKning yuqori ifoda darajasi aniqlangan. MiR-1, miR-208a va miR-423-5p ifodalarining past darajalari lipid darajasi ko'tarilgan yoki gipolipemik dorilarni qo'llagan bemorlarda topilgan.

Xulosa

ST balandligi miokard infarkti bo'lgan bemorlarda MicroRNK ifodasi yurak-qon tomir xavf omillarining mavjudligiga bog'liq.

Kalit so'zlar: yurak-qon tomir xavf omillari, mikroRNK, miokard infarkti.

About 99% of the human genome does not encode proteins but is transcriptionally highly active and give rise to a broad spectrum of non-coding RNAs (ncRNAs) with regulatory and structural functions.

Observation of ncRNAs fraction suggests their important role in humans. One of the many ncRNAs are microRNAs, novel regulators of cardiovascular risk factors and cell functions and thus candidates to improve diagnostic and prognostic assessment [1].

Cardiovascular risk factors contribute to the occurrence of cardiovascular diseases. Classic risk factors include lipid disorders, smoking, hypertension, diabetes, obesity, male sex, low physical activity. In addition, these factors can be divided in two groups: modifiable, which depend on the lifestyle, and non-modifiable, which are not affected by lifestyle or behavior [2].

MicroRNAs are short, single-stranded, non-coding ribonucleic acid molecules that negatively regulate gene expression. During myocardial infarction, microRNAs are released from damaged cardiomyocytes and their level of expression in the blood increases [3,4]. In patients with myocardial infarction presence of cardiovascular risk factors may affect the level of microRNA expression measured in blood.

The aim of this pilot study was to evaluate the expression level of selected microRNAs (miR-1, miR-208a and miR-423-5p) in patients with ST-elevation myocardial infarction with or without cardiovascular risk factors.

Such microRNAs were selected because they are crucial in the development of the heart, generation, and conduction of the stimuli and in the development of cardiovascular diseases (including myocardial infarction) [5,6].

Material & Methods

Seventeen patients with ST-elevation myocardial infarction were included in the study. Age range was 18-85 years old. All patients signed informed consent to participate in this study.

ST-elevation myocardial infarction was diagnosed according to the current guidelines of the European Society of Cardiology [7]. A successful percutaneous coronary angioplasty was performed within six hours of the onset of myocardial infarction pain.

The exclusion criteria from participation in the study were as follows: history of acute coronary syndrome within 30 days prior to enrolment, percutaneous or surgical coronary revascularization within 30 days prior to enrolment, liver insufficiency, renal insufficiency, chronic heart failure, neoplasm, autoimmune disease, chronic obstructive pulmonary disease, active infection, pregnancy, myopathy, history of muscle trauma within 30 days prior to enrolment and lack of informed consent. Cardiovascular risk factors were defined as: arterial hypertension - systolic blood pressure of at least 140 mmHg and/or a diastolic blood pressure of at least 90 mm Hg [10], diabetes mellitus (DM2) – metabolic disease associated with hyperglycemia due to defect in insulin secretion and/or function [11], hyperlipidemia – total cholesterol and/or triglycerides above normal value (cholesterol >190 mg/dl, triglycerides >150 mg/dl) [12], smoking – persistent smoking

[13], positive family history – presence of ischemic heart disease in first degree male relatives under 55y or female relatives under 60y [14].

Blood samples were collected on hospital admission and 6, 12 and 24 hours after coronary angioplasty. Every patient had 5ml of blood acquired using sodium citrate-anti-coagulated specimens from ulnar vein, using the Vacutainer system. After that, blood samples were centrifuged within an hour at 4°C degrees for 10 minutes (1550xg). Supernatant was transferred to RNase / DNase free tubes and stored at -80° C until evaluation. RNA was isolated from these samples using the mirVana Paris Kit from Ambion, according to the manufacturer's instructions. Using the RT-PCR reaction (reverse transcription polymerase chain reaction), the template RNA was transcribed with reverse transcriptase into cDNA (complementary DNA). The material was then multiplied. The TaqMan MicroRNA Assays from Applied Biosystems on the 7900 HT Fast Real-Time PCR System from Applied Biosystems were used to study the expression level of selected microRNAs. The device calculates the value of the threshold cycles. Based on values obtained for the defined initial DNA concentrations, the efficiency of the polymerase chain reaction was determined from the slope of the standard curve. Then, normalization was performed to correct for differences between the compared trials. Test and reference samples were duplicated during normalization. Cel-miR-39 was used in the study. The expression level of the test sample was compared to a calibrator of known expression level [8]. The relative expression level was calculated by the Pfaffel method [9].

Statistical Analysis

Statistical analysis was based on licensed MedCalc software (v. 14.8.1; MedCalc Software bvba, Ostend, Belgium). MicroRNA expression levels are shown as median and interquartile range (IQR; i.e. 25-75 pc). Qualitative variables (male sex, arterial hypertension, diabetes mellitus, positive family history, smoking, hyperlipidemia) were presented as absolute values. Intergroup differences were verified with Mann-Whitney U test. The criterion of statistical significance was established: $p < 0.05$.

Results

Characteristics of patients with STEMI

Twelve men and five women were included in the study. Median age was 57, with the youngest being 40 and the oldest 84. Ten patients were diagnosed with arterial hypertension, nine of them were on pharmacological therapy and had good blood pressure control, seven patients had lipid disorders and used medicaments, eleven patients were smokers, and seven patients had a positive family history. No one were diagnosed with diabetes mellitus.

Table 1

Expression level of miR-1, miR-208a i miR-423-5p in STEMI group

Time	miR-1	miR-208a	miR-423-5p
Baseline	1,16 (1,070-1,542)	1,03 (0,97-1,07)	1,055 (0,995-1,475)
6 h	1,09 (0,935-1,210)	1,02 (0,88-1,08)	1,03 (0,858-1,085)
12 h	1,11 (1,045-1,195)	1,05 (1,01-1,08)	1,05 (0,973-1,072)
24 h	1,12 (1,022-1,165)	1,035 (0,995-1,065)	1,03 (0,955-1,06)

Baseline, ‘6 h’, ’12 h’, ’24 h’ – Time marking points. Median values are shown, the interquartile range is given in brackets.

Table 2

miR-1 expression according to classical cardiovascular risk factors in patients with myocardial infarction

Variable		Time marking points			
		Baseline	**‘6 h’**	**’12 h’**	**’24 h’**
Male sex	YES	1,200 (1,078-1,633)	1,095 (0,95-1,29)	1,125 (1,105-1,235)	1,090 (1,015-1,17)
	NO	1,115 (1,08-1,145)	1,090 (0,92-1,163)	1,050 (0,978-1,183)	1,130 (1,042-1,173)
Arterial hypertension	YES	1,180 (1,06-1,61)	1,090 (0,94-1,12)	1,125 (0,96-1,21)	1,120 (1,00-1,15)
	NO	1,130 (1,095-1,338)	1,150 (0,89-1,40)	1,110 (1,102-1,175)	1,160 (1,06-1,175)
Smoking	YES	1,180 (1,10-1,408)	1,090 (0,875-1,143)	**1,160* (1,11-1,247)**	**1,160* (1,06-1,203)**
	NO	1,130 (1,06-1,81)	1,145 (1,01-1,24)	**0,995 (0,93-1,11)**	**1,075 (0,97-1,12)**

Hyperlipidemia	YES	1,340 (1,085-1,76)	1,090 (0,958-1,23)	1,110 (0,938-1,178)	**1,000* (0,978-1,098)**
	NO	1,130 (1,055-1,19)	1,095 (0,92-1,15)	1,135 (1,10-1,21)	**1,160 (1,13-1,21)**
Positive family history	YES	1,130 (1,10-1,34)	1,090 (0,918-1,187)	1,110 (1,062-1,188)	1,120 (1,038-1,153)
	NO	1,180 (1,048-1,618)	1,055 (0,94-1,24)	1,125 (0,96-1,21)	1,135 (1,00-1,18)

***p<0,05 (for the differences between groups defined by the presence of a risk factor, in the U Mann-Whitney test)**; Baseline, '6 h', '12 h', '24 h' – Time marking points. Median values are shown, the interquartile range is given in brackets.

Evaluation of the differences in the miR-1 expression levels in patients with cardiovascular risk factors and without these factors demonstrated in people with smoking a significantly higher level of miR-1 expression at '12 h' and '24' hour after marking than in non-smokers. On the other hand, patients with a history of hyperlipidemia had a significantly lower level of miR-1 expression at '24' hour than patients without hyperlipidemia.

Table 3

miR-208a expression according to classical cardiovascular risk factors in the group of patients with myocardial infarction

Variable		Time marking points			
		Baseline	**'6 h'**	**'12 h'**	**'24 h'**
Male sex	YES	1,050 (0,99-1,07)	1,020 (0,88-1,22)	1,055 (1,02-1,095)	1,050 (0,992-1,068)
	NO	0,97 (0,97-1,022)	0,97 (0,86-1,07)	1,025 (0,89-1,06)	1,030 (0,953-1,06)
Arterial hypertension	YES	0,99 (0,97-1,078)	1,010 (0,88-1,038)	1,050 (0,925-1,09)	1,030 (0,94-1,075)
	NO	1,035 (1,00-1,055)	1,060 (0,86-1,22)	1,050 (1,02-1,078)	1,040 (1,00-1,058)
Smoking	YES	**1,055* (1,03-1,07)**	0,975 (0,84-1,06)	**1,070* (1,022-1,102)**	1,050 (1,00-1,09)
	NO	**0,97 (0,97-0,985)**	1,045 (0,945-1,15)	**0,93 (0,877-1,05)**	1,005 (0,95-1,03)
Hyperlipidemia	YES	**0,97* (0,97-0,985)**	0,945 (0,845-1,115)	1,03 (0,915-1,05)	**0,975* (0,91-1,01)**
	NO	**1,055 (1,03-1,07)**	1,035 (0,88-1,08)	1,070 (1,02-1,088)	**1,050 (1,03-1,09)**
Positive family history	YES	1,030 (0,985-1,038)	1,060 (0,833-1,115)	1,050 (1,02-1,07)	1,01 (1,00-1,038)

	NO	1,030 (0,97-1,07)	1,010 (0,88-1,085)	1,045 (0,93-1,08)	1,050 (0,98-1,075)

***p<0,05 (for the differences between groups defined by the presence of a risk factor, in the U Mann-Whitney test)**; Baseline, '6 h', '12 h', '24 h' – Time marking points. Median values are shown, in the brackets the interquartile range is given.

When examining the differences of miR-208a expression levels in the myocardial infarction group comparing patients with or without cardiovascular risk factors, it was found that smokers were characterized by significantly higher levels of miR-208a expression at '0 h' and '12' hour than non-smokers. Patients with a history of hyperlipidemia had significantly lower levels of miR-208a expression at '0 h' and '24' hour than those without hyperlipidemia.

Table 4

miR-423-5p expression according to classical cardiovascular risk factors in the group of patients with myocardial infarction

Variable		Time marking points			
		Baseline	**'6 h'**	**'12 h'**	**'24 h'**
Male sex	YES	1,100 (0,988-1,487)	1,025 (0,875-1,17)	1,055 (1,015-1,10)	1,015 (0,94-1,06)
	NO	1,050 (1,022-1,197)	1,030 (0,85-1,062)	0,98 (0,903-1,062)	1,03 (0,938-1,048)
Arterial hypertension	YES	1,060 (0,97-1,262)	1,025 (0,86-1,05)	1,040 (0,95-1,08)	1,015 (0,900-1,06)
	NO	1,050 (1,02-1,588)	1,060 (0,84-1,24)	1,050 (1,012-1,068)	1,030 (0,973-1,058)
Smoking	YES	1,1 (1,02-1,487)	1,00 (0,828-1,058)	**1,06* (1,02-1,11)**	1,050 (0,973-1,06)
	NO	1,050 (0,97-1,175)	1,050 (1,02-1,13)	**0,95 (0,88-1,05)**	0,985 (0,91-1,03)
Hyperlipidemia	YES	1,125 (0,98-1,50)	1,020 (0,895-1,115)	1,020 (0,898-1,058)	**0,910* (0,893-0,992)**
	NO	1,055 (1,01-1,45)	1,030 (0,85-1,06)	1,060 (1,01-1,08)	**1,050 (1,03-1,06)**
Positive family history	YES	1,060 (1,02-1,44)	1,030 (0,865-1,068)	1,050 (0,99-1,068)	1,00 (0,97-1,03)
	NO	1,050 (0,97-1,462)	1,025 (0,86-1,13)	1,04 (0,95-1,08)	1,05 (0,91-1,06)

***p<0,05 (for the differences between groups defined by the presence of a risk factor, in the U Mann-Whitney test)**; '0', '6', '12', '24' – Time marking points. Median values are shown, the interquartile range is given in brackets.

When examining the differences in the levels of miR-423-5p expression between people with and without cardiovascular risk factors in the group of patients with myocardial infarction, it was found that the level of miR-423-5p expression at '12' hour was significantly higher in smokers than in non-smokers. Patients with a history of hyperlipidemia had significantly lower levels of miR-423-5p expression at "24" hour than those not burdened with this risk factor.

Discussion

According to the results of the WOBASZ and NATPOL studies, the prevalence of cardiovascular risk factors in the Polish population is high [15].

In this study, a high frequency of arterial hypertension, hyperlipidemia and nicotinism was observed. Out of 17 subjects, 10 were diagnosed with arterial hypertension, 7 were diagnosed with lipid metabolism disorders, and 11 people were smokers.

The relevance between expression level of the studied microRNAs and the presence of certain cardiovascular risk factors was demonstrated. It has been observed that the expression levels of miR-1, miR-208a and miR-423-5p are higher in smokers than in non-smokers. On the other hand, patients with lipid metabolism disorders were characterized by a lower expression level of the investigated microRNAs compared to people with normal lipid levels. No influence of other cardiovascular risk factors on the expression of studied microRNAs was observed.

There are only a few reports available on this issue.

Ai found no correlation between level of miR-1 expression and sex or diabetes. The abovementioned author studied a large group of 93 patients with STEMI but also NSTEMI. Patients were of a similar age, people under 30 and over 75 were excluded, men dominated. The prevalence of diabetes mellitus was twice as high as in the described study. In addition, Ai presented the miR-1 expression level as a value for threshold cycles[16].

Similarly, Long has shown no correlation between the level of miR-1 expression and sex, diabetes, or smoking. The size of the study group was the same as in this study, what is more, Long qualified not only patients with STEMI but also NSTEMI. Age of the patients was similar, most of them were men. Frequency of the risk factors was different - diabetes occurred twice as often, and cigarette smoking was observed more often than in this study. Nonetheless, Long obtained different results of the miR-1 expression study in patients with myocardial infarction [17].

On the contrary, Gao showed a relationship between some cardiovascular risk factors and the expression of miR-145. He described significantly lower levels of miR-145 expression in patients with diabetes and smoking. Gao studied expression of miR-145 because it is a particle specific to vascular smooth muscle cells, its expression level decreases in people with coronary artery disease. He conducted the study on a much larger group of people and qualified patients with STEMI, NSTEMI, stable and unstable coronary artery disease. The mean age was similar to the median in this analysis, the vast majority were men. The prevalence of cardiovascular risk factors was different. Gao used different methodology and obtained other miR-145 expression level results [18].

Results

This study suggested a correlation between some cardiovascular risk factors and the expression level of the studied microRNAs in patients with myocardial infarction. Higher levels of miRNA expression were observed in smokers than in non-smokers. In patients with increased lipid levels or these using hypolipemic drugs, the expression levels of miR-1, miR-208a and miR-423-5p were lower than in patients with normal lipid levels. There were no differences in the expression levels of the studied microRNAs for sex, hypertension, and positive family history.

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