Am J Transl Res 2021;13(4):3731-3737
www.ajtr.org /ISSN:1943-8141/AJTR0126104

Original Article

The changes in miR-221 and miR-222 before and after interventional therapy of coronary heart disease and analysis of their correlation with inflammatory factors and prognosis

Yunpeng Wang[1*], Haiwen Deng[2*], Zhongshan Xu[2], Pengfei Xu[3], Tiechuan Huo[4,5], Zitao Xu[6,7], Mingwa Guo[2]

[1]The First Department of Cardiology, The Second People's Hospital of Liaocheng, Liaocheng, Shandong Province, China; [2]Department of Cardiology, The Second People's Hospital of Dongying, Dongying, Shandong Province, China; [3]Department of Pharmacy, Yidu Central Hospital, Weifang, Shandong Province, China; Departments of [4]Cardiology, [5]Endocrinology, Quyang County Hospital of Traditional Chinese Medicine, Quyang, Hebei Province, China; Departments of [6]Cardiology, [7]Intensive Care Unit, China Water Resource and Hydropower No. 13th Engineering Bureau Hospital, Dezhou, Shandong Province, China. [*]Equal contributors and co-first authors.

Received November 12, 2020; Accepted December 23, 2020; Epub April 15, 2021; Published April 30, 2021

Abstract: Objective: To explore the changes in miR-221 and miR-222 before and after interventional therapy of coronary heart disease and their relationship with inflammatory factors and prognosis. Methods: A total of 122 subjects with coronary heart disease who underwent interventional therapy in our hospital from January 2017 to January 2019 were chosen as the observation group, and 122 healthy people during the same period were chosen as the control group. We retrospectively analyzed the levels of serum miR-221, miR-222, C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Pearson correlation analysis was used to reveal the relationship between serum levels of miR-221, miR-222 and CRP, TNF-α and IL-6, N-terminal B-type brain natriuretic peptide precursor (NT-proBNP) and left ventricular ejection fraction (LVEF%) in the observation group. The levels of serum miR-221, miR-222, TNF-α, CRP and IL-6 before and after treatment were compared in the observation group. After a follow-up of 6 months, the observation group was divided into a poor-prognosis group (26 cases) and a good-prognosis group (96 cases) according to whether there was an adverse cardiovascular event or not. The levels of serum miR-221 and miR-222 before and after intervention treatment were compared between the two groups. And the clinical values of miR-221 and miR-222 levels before and after intervention treatment in the observation group were analyzed by the ROC curve. Results: The levels of serum miR-221, miR-222, CRP, TNF-α, and IL-6 in the observation group were markedly higher than those of the control group. And levels of serum miR-221, miR-222 were negatively correlated with LVEF% while positively correlated with CRP, TNF-α, IL-6 and NT-proBNP ($P<0.05$). After treatment, the levels of miR-221, miR-222, CRP, TNF-α, and IL-6 in the observation group were significantly reduced (all $P<0.05$). Compared with the good prognosis group, the levels of miR-221 and miR-222 before and after treatment were markedly higher in the poor prognosis group (all $P<0.05$). Both before and after treatment, the levels of miR-221 and miR-222 have certain clinical value in evaluating the prognosis (all AUC>0.800). Conclusion: The levels of miR-221 and miR-222 in patients with coronary heart disease significantly increased and they were closely correlated with the inflammatory factors, NT-proBNP and LVEF%. The levels of miR-221 and miR-222 before and after treatment have certain clinical value in evaluating the prognosis of patients.

Keywords: miR-221, miR-222, coronary heart disease, intervention, inflammatory factors

Introduction

Coronary heart disease is a type of common cardiovascular and cerebrovascular diseases caused by atherosclerosis (AS). Its basic pathological principle is coronary AS, which promotes the accumulation of lipid on the intima of the artery to form plaques. A large increase of plaques will further narrow or even block the arterial lumen, resulting in blocked blood flow, myocardial ischemia, hypoxia, or necrosis, which can lead to angina pectoris [1-3]. It has been

shown that coronary heart disease has a high disability rate and mortality rate. Meanwhile, its incidence rate is also rising, which seriously affects human health [4]. At present, a main clinical therapy for coronary heart disease is percutaneous coronary intervention (PCI). However, because of the complexity of disease conditions and the presence of comorbidities, the prognosis of coronary heart disease is poor. Researchers have found that about 35% of patients will still have clinical manifestations of cardiac ischemia in the next 5 years [5-7]. Therefore, we explored whether there is a specific biomarker contributing to the diagnosis, treatment, and prognosis prediction of coronary heart disease. MicroRNA (miR) is a group of endogenous non-coding small RNA molecules, which are specifically expressed in cardiomyocytes and are closely related to the occurrence and development of cardiovascular diseases such as myocardial infarction, heart failure, and arrhythmia [8-10]. miR-221/miR-222 is widely present in human cells and tissues and is specifically highly expressed in endothelial cells. miR-221 and miR-222 have been closely associated with the occurrence and development of tumors and may also be involved in the process of AS and ischemic cardiovascular and cerebrovascular diseases [11, 12]. However, there are still few reports on its changes before and after coronary intervention and its roles in inflammatory response and prognosis evaluation. Here, we aim to provide relevant evidence for clinical evaluation of prognosis by exploring the changes of miR-221 and miR-222 before and after intervention therapy of coronary heart disease and their correlation with inflammatory factors and prognosis.

Materials and methods

Subject

One hundred and twenty-two patients with coronary heart disease who received interventional treatment in The Second People's Hospital of Liaocheng from January 2017 to January 2019 were chosen as the observation subjects, and 122 healthy people were chosen as the control group during the same period. All research subjects and their families had agreed and signed the consent form. This study was approved by the Ethics Committee of The Second People's Hospital of Liaocheng.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients meeting the diagnostic criteria for coronary heart disease [13]. (2) Patients who met the indications for interventional therapy and received interventional therapy [13]. (3) Patients of whom the onset time ≤12 h; (4) patients without interventional therapy before.

Exclusion criteria: (1) those who have undergone surgery before; (2) patients with severe important organ dysfunction; (3) those who were pregnant or lactating; (4) those with neurological or mental abnormalities; (5) those who may have other acute inflammatory reaction diseases in the near future.

Methods

When the observation group was enrolled, the left ventricular ejection fraction (LVEF%) of the two groups of patients before and after treatment was detected by ACUSONS2000 color doppler diagnostic instrument (Siemens, Germany). For the control group, 2 tubes of 5 mL venous blood were took in the next morning before breakfast. For the observation group, 2 tubes of 5 mL venous blood were took 2 h before operation and 24 h after operation. One tube was centrifuged at 3000 r/min for 5 min to separate the serum. Chemiluminescence method (Elecss-2010 electrochemiluminescence immunoassay analyzer, Roche) was used to evaluate the level of N-terminal B-type brain natriuretic peptide precursor (NT-proBNP). Immunoturbidimetry (Roche/Hitachi cobasc501 analyzer, Swiss Roche) was used to detect the level of C-reactive protein (CRP), and enzyme-linked immunosorbent assay (Spectra-Max Paradigm multifunctional microplate reader, Molecular Devices, USA) was used to detect the level of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). For another tube, RNA extraction kit was used to extract peripheral blood RNA. Then reverse transcription reaction was performed according to reverse transcription kit, and RT-PCR experiment was carried out according to the instructions of SYBR RT-PCR kit (Hangzhou Bori Technology Co., Ltd., China). The reaction system was listed as follows: predenaturation at 95°C for 30 s, 95°C for 5 s, 60°C for 30 s, and 35 cycles. The relative expression level of miR-221 and miR-222 in

Table 1. Primer sequences

Primers	Sequences
miR-221 upstream	GGGATGAACCTGGCATACAA
miR-221 downstream	CTACCTGGAAACATGTTCTC
miR-222 upstream	GTTCGTGGGAGCTACATCTGGC
miR-222 downstream	GTGTCGTGGAGTCGGCAATTC
U6 upstream	CTCGCTTCGGCAGCACA
U6 downstream	AACGCTTCACGAATTTGCGT

Table 2. General information of two groups (n, ($\bar{x}$ ± sd))

Characteristics	Observation group (n=122)	Control group (n=122)	t/χ^2	P
Age (years)	58.3±6.8	57.8±7.1	0.562	0.575
Gender (n)			0.068	0.795
Male	71	73		
Female	51	49		
BMI (kg/m^2)	22.73±3.12	22.91±3.42	0.480	0.632
Systolic BP (mmHg)	129.11±14.47	129.83±13.94	0.386	0.700
Diastolic BP (mmHg)	74.52±11.46	75.11±12.34	0.395	0.693
Heart rate (bpm)	73.42±9.42	73.15±9.21	0.252	0.801
Smoking history (n)			0.082	0.775
Yes	35	33		
No	87	89		
Drinking history (n)			0.170	0.680
Yes	31	28		
No	91	93		
Hypertension history (n)			0.166	0.683
Yes	42	39		
No	80	83		

each group was measured by $2^{-\Delta\Delta Ct}$ method. The internal reference was U6. Each sample was repeated three times and the average value was taken. The primers were synthesized by Beijing Dingguo Biotechnology Co, Ltd., China. The sequences of primers were shown in **Table 1**. The observation group was followed up 6 months after surgery, and the observation group was divided into a poor-prognosis group and a good-prognosis group according to whether there were adverse events. Adverse events were defined as cardiovascular events such as stent thrombosis, restenosis, acute myocardial infarction, or death [13].

Outcome measures

Serum miR-221, miR-222, CRP, TNF-α and IL-6 levels in two groups were analyzed. Pearson correlation method was adopted to evaluate the relation between serum levels of miR-221, miR-222 and CRP, TNF-α, IL-6, NT-ProBNP, and LVEF% in the observation group. The levels of miR-221, miR-222, TNF-α, CRP and IL-6 in the observation group were compared before and after treatment. The levels of miR-221 and miR-222 before and after treatment were compared between the poor-prognosis group and the good-prognosis group. The clinical value of miR-221 and miR-222 levels before and after treatment in the observation group was evaluated by ROC curve.

Statistical analysis

We adopted SPSS 22.0 to carry out statistical analysis. The numeration data were represented by the number of cases and percentage (n, %), and those in accordance with the normal distribution were represented by the mean ± standard deviation ($\bar{x}$ ± sd). In addition, the t-test was adopted to compare the measurement data between groups, and the χ^2 test was adopted to compare the numeration data. Meanwhile, Pearson Correlation Coefficient was used for correlation analysis. For the observation group, ROC curve was drawn to evaluate the clinical value of miR-221 and miR-222 levels before and after treatment in evaluating prognosis. $P<0.05$ was regarded as statistically significant.

Results

Characteristics

It was showed that no statistically significant difference existed between the two groups in terms of age, gender, and other general characteristics (all $P>0.05$). So, these two groups were comparable. For details, see **Table 2**.

Table 3. Comparison of the levels of inflammatory factors, miR-221 and miR-222 ($\bar{x} \pm sd$)

Groups	CRP (mg/L)	IL-6 (ng/L)	TNF-α (ng/L)	miR-221	miR-222
Observation group (n=122)	9.17±1.24	7.76±1.43	9.58±1.27	7.02±2.58	6.17±2.51
Control group (n=122)	0.48±0.21	3.22±0.81	3.08±0.64	1.01±0.24	1.04±0.22
t	76.320	30.512	50.483	25.619	22.489
p	<0.001	<0.001	<0.001	<0.001	<0.001

Note: CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha.

Table 4. Correlation between the levels of miR-221 and miR-222 and the levels of various indicators (r)

Indicators	CRP (mg/L)	IL-6 (ng/L)	TNF-α (ng/L)	NT-proBNP (pg/mL)	LVEF%
miR-221					
r	0.287	0.319	0.326	0.394	-0.293
P	<0.001	0.012	<0.001	<0.001	<0.001
miR-222					
r	0.315	0.295	0.275	0.288	-0.234
P	0.003	<0.001	<0.001	0.007	<0.001

Note: CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; NT-proBNP: N-terminal B-type brain natriuretic peptide precursor; LVEF%: left ventricular ejection fraction.

Table 5. Changes in the levels of indicators before and after treatment ($\bar{x} \pm sd$)

Groups	CRP (mg/L)	IL-6 (ng/L)	TNF-α (ng/L)	miR-221	miR-222
Before treatment	9.17±1.24	7.76±1.43	9.58±1.27	7.02±2.58	6.17±2.51
After treatment	5.81±1.98	3.44±1.85	6.02±1.23	4.69±2.40	3.83±2.17
t	15.886	20.407	22.241	7.304	7.790
p	<0.001	<0.001	<0.001	<0.001	<0.001

Note: CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha.

Table 6. Comparison of miR-221 and miR-222 levels between the poor-prognosis group and the good-prognosis group before and after treatment ($\bar{x} \pm sd$)

Groups	miR-221		miR-222	
	Before treatment	After treatment	Before treatment	After treatment
Poor-prognosis group (n=26)	9.99±2.82	7.80±2.57	8.66±3.02	6.50±2.54
Good-prognosis group (n=96)	6.22±1.82	3.85±1.50	5.50±1.87	3.11±1.35
t	8.256	10.053	6.623	9.188
P	<0.001	<0.001	<0.001	<0.001

Comparison of the levels of inflammatory factors, miR-221 and miR-222 between the two groups

Compared with the control group, the levels of CRP, IL-6, TNF-α, miR-221 and miR-222 in the observation group were significantly higher (all P<0.001), as shown in **Table 3**.

Correlation between the levels of miR-221, miR-222 and the levels of various indicators in the observation group

In the observation group, the levels of miR-221 and miR-222 were negatively correlated with LVEF%, while positively correlated with CRP, TNF-α, IL-6, and NT-proBNP (all P<0.05), as shown in **Table 4**.

Changes in the levels of indicators before and after treatment in the observation group

After treatment, the levels of miR-221, miR-222, CRP, TNF-α, and IL-6 in the observation group decreased (all P<0.05), as shown in **Table 5**.

Comparison of miR-221 and miR-222 levels between the poor-prognosis group and the good-prognosis group before and after treatment

Compared with the good-prognosis group, the levels of miR-221 and miR-222 before and after treatment were markedly higher in the poor-prognosis group (all P<0.05). The results were displayed in **Table 6**.

Results of ROC curve

Results of ROC curve showed that in the observation group, the levels of miR-221 and miR-

Table 7. Results of ROC curve

Indicators	Cut-off point	AUC	95% CI	Sensitivity	Specificity
miR-221 (Before treatment)	7.341	0.873	0.797, 0.948	0.846	0.823
miR-221 (After treatment)	5.099	0.918	0.862, 0.974	0.885	0.854
miR-222 (Before treatment)	6.469	0.812	0.718, 0.907	0.769	0.792
miR-222 (After treatment)	4.211	0.898	0.837, 0.960	0.846	0.844

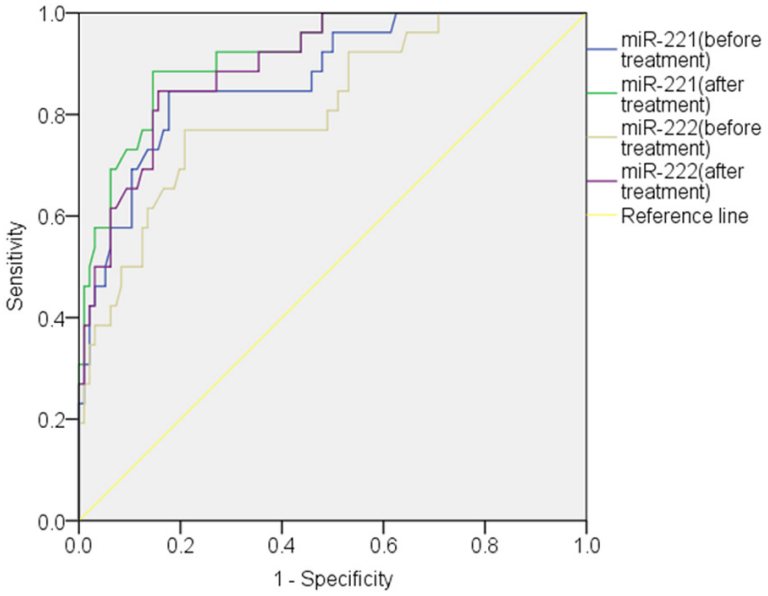

Figure 1. ROC curve results.

222 before and after treatment had certain clinical value in evaluating the prognosis (all AUC>0.800). **Table 7** and **Figure 1** showed the results.

Discussion

MiRNA participates in biological processes, such as cell differentiation and proliferation, and its abnormalities are often closely related to various diseases, such as coronary heart disease and heart failure. Researches have revealed that miRNA influences the occurrence and development of coronary heart disease by participating in gene regulation of major cell functions [14, 15]. Therefore, exploring the expression of miRNA in patients with coronary heart disease is vital to the diagnosis, treatment, and prognosis of coronary heart disease. MiR-221/222 are two miRNAs with the same sequence, which are involved in angiogenesis, blood cell growth, malignant tumor development and other processes, and are abnormally expressed in various diseases [16]. Endothelial injury is regarded as the major risk factor for the progress of AS. Endothelial progenitor cells (EPCs) can maintain endothelial homeostasis and achieve vascular repair through reendothelialization and angiogenesis. The decrease of EPCs in the body can cause vascular inflammation.

In this study, we found that the amount of EPCs in patients with coronary heart disease is negatively correlated with the level of miR-221/222. MiR-221/222 can mediate multiple target genes, such as c-kit, E-box binding zinc finger protein 2, transcription factor ETs1/2 etc., to play an anti-angiogenic effect [17]. The arterial intima is mainly composed of VSMC, and VSMC can affect wound healing, vascular injury, and other processes by mediating vascular proliferation. During intimal hyperplasia, VSMC proliferates actively, and thickens the arterial wall by migrating to the intima. The abnormal proliferation of VSMC is often closely related to the formation of AS. It was found in a previous study that miR-221/222 is overexpressed in VSMC, which could promote the proliferation of VSMC and inhibit the function of EPCs to play a role [18]. In this study, it was showed that compared with the normal population, the level of miR-221/222 in patients with coronary heart disease increased, and it was closely related to LVEF% and NT-proBNP. However, after interventional therapy, the level of it has decreased. Compared with patients with good prognosis, patients with poor prognosis still showed a higher level of miR-221/222.

AS is considered to be the main pathological basis for the development of coronary heart disease, and the essence of AS is a chronic

inflammatory process. In the present study, we found that the inflammatory response was involved in the occurrence and development of AS and thrombosis [19, 20]. At present, PCI surgery has been widely used in the treatment of coronary heart disease. While improving coronary artery disease, it may also cause intimal detachment, tearing, plaque rupture, etc., which may lead to damage and inflammatory reaction of the blood vessel wall, resulting in the abnormal structure and function of vascular endothelium. It can even cause myocardial damage and necrosis in severe cases. CRP is a type of acute phase inflammatory response protein, which have characteristics of early appearance and rapid rising and sensitively reflect the body's inflammation and stress state. When an infectious disease or inflammatory reaction occurs in the body, CRP levels will rise to varying extents [21]. IL-6 contributed greatly to the development of atherosclerosis. It could be caused by humoral immunity, cellular immunity, etc., which leads to tissue function damage and even AS. Meanwhile, it also has a certain correlation with plaque stability. TNF-α is mainly produced by macrophages and monocytes and has a wide range of biological properties. The level of TNF-α in the body is positively correlated with carotid intima thickness and age-related AS and is involved in the formation of AS. The possible mechanism is promoting LDL-C overexpression and reducing the level of HDL-C. At the same time, it can also induce the apoptosis of vascular endothelial cells and stimulate the expression of vascular endothelial factors, causing abnormal vascular endothelial function and the formation of thrombosis. The results of this study showed that the levels of CRP, IL-6 and TNF-α in patients with coronary heart disease were significantly higher than those in the normal population. After treatment, the inflammatory response in patients with coronary heart disease was reduced. Studies have confirmed that miRNA is closely related to the AS-mediated inflammatory response process. Meanwhile, Other studies have found that during the AS inflammatory response involving macrophages, miR-21, miR-210, and miR-147 increased significantly [22]. It was revealed in this study that miR-221/222 was positively correlated with the levels of inflammatory factors such as CRP, IL-6 and TNF-α in patients with coronary heart disease, which also suggested that miR-221/222 could be involved in the inflammatory process mediated by patients with coronary heart disease. The study further explored the evaluation value of miR-221/222 in the prognosis of patients with coronary heart disease. The results showed that before and after treatment, the level of miR-221/222 has certain clinical value in evaluating the prognosis of patients with coronary heart disease. It also provided new ideas for the clinical evaluation of postoperative prognosis.

In summary, the levels of miR-221 and miR-222 in patients with coronary heart disease increased significantly, and those indicators were closely related to inflammation factors, NT-proBNP, and LVEF%. Moreover, the levels of miR-221 and miR-222 before and after treatment have certain clinical values in evaluating the prognosis of patients. However, there were still some limitations in this study, such as non-dynamic observation of the changes of various indicators, single sample source, single center, etc. Thus, it needs further investigation.

Disclosure of conflict of interest

None.

Address correspondence to: Mingwa Guo, Department of Cardiology, The Second People's Hospital of Dongying, No. 28 Changchun Road, Dawang Town, Guangrao County, Dongying 257335, Shandong Province, China. Tel: +86-0546-6625663; E-mail: guomingwa2ndy@163.com

References

[1] Liu ZX, Ji HH, Yao MP, Wang L, Wang Y, Zhou P, Liu Y, Zheng XF, He HW, Wang LS, Gao W and Lu X. Serum metrnl is associated with the presence and severity of coronary artery disease. J Cell Mol Med 2019; 23: 271-280.

[2] Gisterå A and Hansson GK. The immunology of atherosclerosis. Nat Rev Nephrol 2017; 13: 368-380.

[3] Moriya J. Critical roles of inflammation in atherosclerosis. J Cardiol 2019; 73: 22-27.

[4] Schnatz PF, Jiang X, Aragaki AK, Nudy M, O'Sullivan DM, Williams M, LeBlanc ES, Martin LW, Manson JE, Shikany JM, Johnson KC, Stefanick ML, Payne ME, Cauley JA, Howard BV and Robbins J. Effects of calcium, vitamin d, and hormone therapy on cardiovascular disease risk factors in the women's health initiative: a randomized controlled trial. Obstet Gynecol 2017; 129: 121-129.

[5] Farrokhian A, Raygan F, Bahmani F, Talari HR, Esfandiari R, Esmaillzadeh A and Asemi Z. Long-term vitamin D supplementation affects metabolic status in vitamin D-deficient type 2 diabetic patients with coronary artery disease. J Nutr 2017; 147: 384-389.

[6] Ali ZA, Gao R, Kimura T, Onuma Y, Kereiakes DJ, Ellis SG, Chevalier B, Vu MT, Zhang Z, Simonton CA, Serruys PW and Stone GW. Three-year outcomes with the absorb bioresorbable scaffold: individual-patient-data meta-analysis from the ABSORB randomized trials. Circulation 2018; 137: 464-479.

[7] Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, Byrne RA, Collet JP, Falk V, Head SJ, Jüni P, Kastrati A, Koller A, Kristensen SD, Niebauer J, Richter DJ, Seferovic PM, Sibbing D, Stefanini GG, Windecker S, Yadav R and Zembala MO. 2018 ESC/EACTS guidelines on myocardial revascularization. the task force on myocardial revascularization of the european society of cardiology (ESC) and european association for cardiothoracic surgery (EACTS). G Ital Cardiol (Rome) 2019; 20: 1S-61S.

[8] Ludwig N, Leidinger P, Becker K, Backes C, Fehlmann T, Pallasch C, Rheinheimer S, Meder B, Stähler C, Meese E and Keller A. Distribution of miRNA expression across human tissues. Nucleic Acids Res 2016; 44: 3865-3877.

[9] Martens CR, Bansal SS and Accornero F. Cardiovascular inflammation: RNA takes the lead. J Mol Cell Cardiol 2019; 129: 247-256.

[10] Ojha R, Nandani R, Pandey RK, Mishra A and Prajapati VK. Emerging role of circulating microrna in the diagnosis of human infectious diseases. J Cell Physiol 2019; 234: 1030-1043.

[11] Bazan HA, Hatfield SA, Brug A, Brooks AJ, Lightell DJ Jr and Woods TC. Carotid plaque rupture is accompanied by an increase in the ratio of serum circR-284 to miR-221 levels. Circ Cardiovasc Genet 2017; 10.

[12] Coskunpinar E, Cakmak HA, Kalkan AK, Tiryakioglu NO, Erturk M and Ongen Z. Circulating miR-221-3p as a novel marker for early prediction of acute myocardial infarction. Gene 2016; 591: 90-96.

[13] Wang Y, Liu HH, Li L, Yang WY, Hong T and Luan H. Cardiovascular disease diagnosis and treatment guidelines. Beijing: Military Med Sci Press; 2010.

[14] Ojha R, Nandani R, Pandey RK, Mishra A and Prajapati VK. Emerging role of circulating microRNA in the diagnosis of human infectious diseases. J Cell Physiol 2019; 234: 1030-1043.

[15] Fan R, Xiao C, Wan X, Cha W, Miao Y, Zhou Y, Qin C, Cui T, Su F and Shan X. Small molecules with big roles in microRNA chemical biology and microRNA-targeted therapeutics. RNA Biol 2019; 16: 707-718.

[16] Castagna MG, Marzocchi C, Pilli T, Forleo R, Pacini F and Cantara S. MicroRNA expression profile of thyroid nodules in fine-needle aspiration cytology: a confirmatory series. J Endocrinol Invest 2019; 42: 97-100.

[17] Chistiakov DA, Sobenin IA and Orekhov AN. Vascular extracellular matrix in atherosclerosis. Cardiol Rev 2013; 21: 270-288.

[18] Liu X, Cheng Y, Yang J, Xu L and Zhang C. Cell-specific effects of miR-221/222 in vessels: molecular mechanism and therapeutic application. J Mol Cell Cardiol 2012; 52: 245-255.

[19] Gordon SM and Remaley AT. High density lipoproteins are modulators of protease activity: implications in inflammation, complement activation, and atherothrombosis. Atherosclerosis 2017; 259: 104-113.

[20] Otsuka F, Yasuda S, Noguchi T and Ishibashi-Ueda H. Pathology of coronary atherosclerosis and thrombosis. Cardiovasc Diagn Ther 2016; 6: 396-408.

[21] Stirling AD, Moran NR, Kelly ME, Ridgway PF and Conlon KC. The predictive value of C-reactive protein (CRP) in acute pancreatitis is interval change in CRP an additional indicator of severity. HPB (Oxford) 2017; 19: 874-880.

[22] Raitoharju E, Lyytikäinen LP, Levula M, Oksala N, Mennander A, Tarkka M, Klopp N, Illig T, Kähönen M, Karhunen PJ, Laaksonen R and Lehtimäki T. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the tampere vascular study. Atherosclerosis 2011; 219: 211-217.