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Original Article

Predictive value of miR-374a-5p of peripheral blood mononuclear cells in deep venous thrombosis for elderly patients after total hip arthroplasty

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Abstract: Objective: This study analyzed the predictive value of peripheral blood mononuclear cells' (PBMCs) miR-374a-5p in deep vein thrombosis (DVT) after total hip arthroplasty in the elderly. Methods: There was a total of 112 elderly patients that underwent elective total hip arthroplasty in our hospital and were enrolled as the research subjects. According to the color Doppler ultrasound of lower limbs 5 d postoperatively, subjectswere classified into thrombosis group (n=31) and non-thrombosis group (n=81). We detected the expression levels of miR-374a-5p in PBMCs and plasma D-dimer of the two groups of patients 1 d before surgery, and 1 d, 3 d, and 5 d after surgery, and analyzed the predictive value, and the correlation between miR-374a-5p and plasma D-dimer for DVT by ROC curve. Results: The relative expression of miR-374a-5p in PBMCs and plasma D-dimer of the two groups 1 d, 3 d, and 5 d after surgery were higher than those on the 1 d before surgery (*P<0.05*), and the indexes of thrombus-group-patients were higher than those in the non-thrombosis group at various time points after surgery (*P<0.05*). We analyzed the predictive value of miR-374a-5p for DVT by ROC curve, and the areas under the predictive curve of miR-374a-5p on 1 d pre-surgery, and 1 d, 3 d and 5 d post-surgery for DVT were 0.435, 0.820, 0.974 and 0.782 respectively. The areas under the predictive curve of D-dimer for DVT was 0.470, 0.834, 0.863 and 0.790 respectively on 1 d pre-surgery, and 1 d, 3 d and 5 d post-surgery. The correlation analysis indicated that miR-374a-5p was extremely positively correlated with D-dimer (*P<0.05*). Conclusion: miR-374a-5p is of important value in the prediction of DVT in elderly patients with total hip arthroplasty. Its expression level has a critically positive correlation with D-dimer degree, which is likely to be abiologic indicator for the early diagnosis and treatment of DVT.

Keywords: Peripheral blood mononuclear cells (PBMCs), miR-374a-5p, elderly, total hip arthroplasty, deep vein thrombosis

Introduction

Hip joint disease is one of the most frequent orthopedic diseases in the elderly, and damage to it can cause serious affects to daily life [1]. Hip arthroplasty is a common surgical method used clinically to treat hip diseases. However, deep vein thrombosis (DVT) of lower extremities often occurs due to the large surgical trauma caused by hip replacement and the necessity for bed-rest after surgery [2, 3]. For severe cases, the thrombus may separate to cause pulmonary embolism, which endangers the patient's life. Studies have proposed that early diagnosis and treatment of lower extremity thrombosis can effectively reduce the incidence of pulmonary embolism, and is of significant value in improving the treatment prognosis [4, 5]. miRNA is a type of highly conserved non-coding single-stranded RNA (around 22 bases). It binds to mRNA by base pairing, and regulates protein expression by inhibiting mRNA translation [6]. miRNAs can exist stably in the systemic circulatory system. Scholars consider that the circulating miRNAs may be a new biomarker for DVT in elderly patients with lower limb surgery [7, 8]. In order that the early diagnosis of DVT in elderly patients after total hip arthroplasty can be further improved, this study explored and analyzed the miR-374a-5p in PBMCs for predicting DVT in elderly patients after total hip arthroplasty.

Cases and methods

Research subjects

During May 2018 to May 2020, there was a total of 112 elderly patients that underwent elective total hip arthroplasty in our hospital and were enrolled as the research subjects. According to color Doppler ultrasound of lower limbs 5 d postoperatively, subjectswere classified into a thrombosis group (n=31) and non-thrombosis group (n=81). The research had passed the approval of the ethics committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria: (1) All objects enrolled were first time recipients of total hip arthroplasty; (2) The patient had no preoperative experience or family history of DVT; (3) The patient had no abnormal coagulation mechanism, and hemoglobin was over 100 g/L; (4) Patient with negative detection result of preoperative deep vein doppler ultrasound; (5) Patient aged over 60 years old; (6) No anticoagulant drugs were taken before enrollment; and (7) Patients voluntarily signed the agreement.

Exclusion criteria: (1) Patient with bleeding tendency; (2) Patients who experienced lower extremity surgery before; or (3) Patients with severe organ dysfunction.

Methods

All the patients underwent lateral total hip arthroplasty by our medical team. DVT was confirmed by color Doppler ultrasonography of deep veins in both lower extremities 1 d before and 5 d after surgery. And the fasting venous blood in early morning was extracted 1 d before surgery, and 1 d, 3 d and 5 d after surgery.

miR-374a-5p expression in PBMCs: We used a human peripheral blood lymphocyte separator (purchased from Beijing Solarbio Technology Co., Ltd.) to separate PBMCs, extracted the total RNA by Trizol method, and determined the purity and concentration via ultraviolet spectrophotometer. The cDNA of miRNA was synthesized in line with the instructions of miRNA 1st Strand cDNA Synthesis Kit (purchased from TaKaRa, Japan), and subjected to PCR according to the kit procedures. The PCR reaction conditions were as follows: to conduct pre-denaturation at 95°C for 5 min, denaturation at 95°C for 40 s, annealing at 60°C for 20 s, and extension at 72°C for 15 s. The reaction was repeated for 40 consecutive times. The forward and reverse primer of miR-374-5p were 5'-GCCGGTTATAATACAACCTGATAAG-3' and 5'-TATGGTTGTTCTCTGCTCTC-3'; and for U6 were 5'-CAGCACATATACTAAAATTGGAACG-3' and 5'-ACGAATTTGCGTGTCATCC-3' respectively. By taking U6 as the internal reference gene, the relative expression of miR-374-5p was calculated by $2^{-\Delta\Delta CT}$ method.

Level of plasma D-dimer: We collected peripheral venous blood from patients, centrifuged it, and separated the plasma. The plasma D-dimer level was detected on the SYSMEX CA7000 automatic coagulation analyzer by kit and supporting quality control provided by SIMENS.

Statistical analysis

Data processing and analysis were conducted by SPSS 23.0. The comparison of measurement data was by *t*-test, and the comparison of enumeration data was by χ^2 test. We applied the ROC method to analyze the predictive value of miR-374-5p and D-dimer on DVT, and Pearson correlation analysis to analyze the correlation. *P<0.05* was considered as significant. The graphic software was by SPSS 23.0 and Graphpad prism9.

Results

Clinical data

The differences in gender, age, BMI, intraoperative blood loss, and length of operation between the two groups of patients were not significant (*P>0.05*), as shown in **Table 1**.

Comparison of miR-374a-5p expression between two groups

The relative expression level of miR-374a-5p in PBMCs of the two groups 1 d, 3 d and 5 d postoperatively were higher than that 1 d before surgery [(1.628±0.283), (1.975±0.231), (1.593±0.328) vs. (1.053±0.103); (1.319±0.216), (1.419±0.226), (1.308±0.294) vs. (1.071±0.095)] *(P<0.05*), and the indexes of thrombosis-group-patients were much higher than those in the non-thrombosis-group at each time point [(1.628±0.283) vs. (1.319±0.216); (1.975±0.231) vs. (1.419±0.226); (1.593±0.328) vs. (1.308±0.294)] (*P<0.05*) (**Table 2**).

Table 1. Comparison of clinical data between the two groups

Clinical data	Thrombus-group (n=31)	Non-thrombotic-group (n=81)	t/χ^2	P
Gender				
Male	17	35	1.219	0.270
Female	14	46		
Age (yd, $\bar{x}\pm s$)	69.48±7.42	69.10±8.33	0.222	0.825
BMI (kg/m^2, $\bar{x}\pm s$)	23.95±4.16	24.17±3.46	0.284	0.777
Intraoperative blood loss (ml, $\bar{x}\pm s$)	218.95±45.50	201.58±52.42	1.625	0.107
Length of operation (min, $\bar{x}\pm s$)	73.28±16.50	72.19±17.63	0.298	0.766

Table 2. Comparison of miR-374a-5p expression between the two groups ($\bar{x}\pm s$)

Group	Number of cases	1 d before surgery	1 d after surgery	3 d 1 d after surgery	5 d 1 d after surgery
Thrombus-group	31	1.053±0.103	1.628±0.283*	1.975±0.231*	1.593±0.328*
Non-thrombotic-group	81	1.071±0.095	1.319±0.216*	1.419±0.226*	1.308±0.294*
t	-	0.876	6.195	11.578	4.444
P	-	0.383	0.031	<0.001	0.005

Note: Compared with the same group 1 d before surgery, *$P<0.05$.

Table 3. Comparison of plasma D-dimer levels between two groups of patients (mg/L, $\bar{x}\pm s$)

Group	Number of cases	1 d before surgery	1 d after surgery	3 d 1 d after surgery	5 d 1 d after surgery
Thrombus-group	31	1.45±0.46	4.39±1.07*	6.58±2.10*	4.57±1.49*
Non-thrombotic-group	81	1.53±0.39	3.13±0.89*	4.39±1.54*	3.12±1.36*
t	-	0.923	6.330	6.061	4.916
P	-	0.358	<0.001	<0.001	<0.001

Note: Compared with the same group 1 d before surgery, *$P<0.05$.

Comparison of D-dimer levels in plasma between two groups

The relative expression of plasma D-dimer in the two groups 1 d, 3 d, and 5 d postoperatively was evidently higher than that 1 d before surgery [(4.39±1.07), (6.58±2.10), (4.57±1.49) vs. (1.45±0.46); (3.13±0.89), (4.39±1.54), (3.12±1.36) vs. (1.53±0.39)] (*$P<0.05$*), and the thrombus-group-patients had higher relative expression of plasma D-dimer than non-thrombus-group at each time point after surgery [(4.39±1.07) vs. (3.13±0.89); (6.58±2.10) vs. (4.39±1.54); (4.57±1.49) vs. (3.12±1.36)] (*$P<0.05$*). The details are listed in **Table 3**.

Predictive value of miR-374a-5p for DVT by ROC curve analysis

We applied ROC curve to analyze the predictive value of miR-374a-5p for DVT. The areas under the prediction curve of miR-374a-5p on 1 d pre-surgery, and 1 d, 3 d, and 5 d post-surgery for DVT were 0.435 (95% CI: 0.317~0.553, P=0.288), 0.820 (95% CI: 0.722~0.918, P< 0.001) 0.974 (95% CI: 0.946~1.000, P<0.001) and 0.782 (95% CI: 0.685~0.879, P<0.001) respectively (see **Figure 1**).

Predictive value of D-dimer for DVT by ROC curve analysis

The area under the predictive curve of D-dimer for DVT was 0.470 (95% CI: 0.341~0.599, P=0.628) 0.834 (95% CI: 0.746~0.921, P< 0.001), 0.863 (95% CI: 0.785~0.940, P< 0.001) and 0.790 (95% CI: 0.697~0.882, P<0.001) respectively on 1 d pre-surgery, and 1 d, 3 d and 5 d post-surgery, as in **Figure 2**.

Analysis of correlation between miR-374a-5p and D-dimer

The correlation analysis indicated that miR-374a-5p was extremely positively correlated

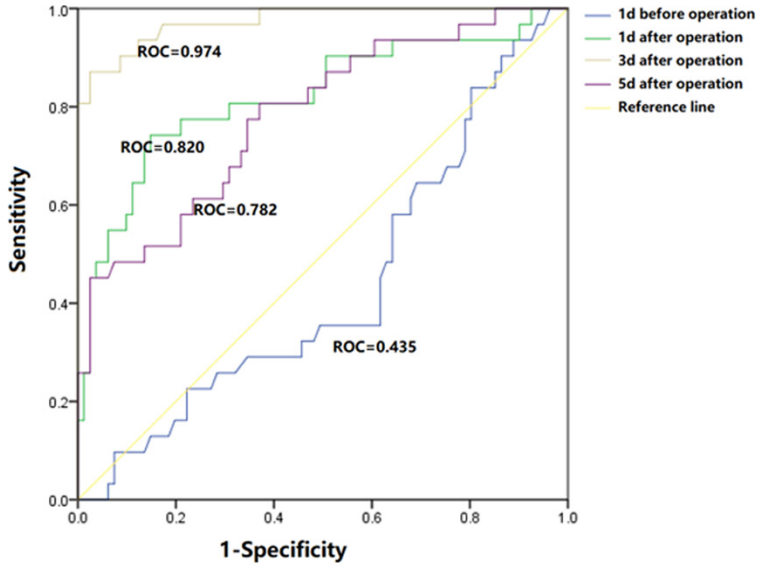

Figure 1. ROC curve analysis of the predictive value of miR-374a-5p for DVT.

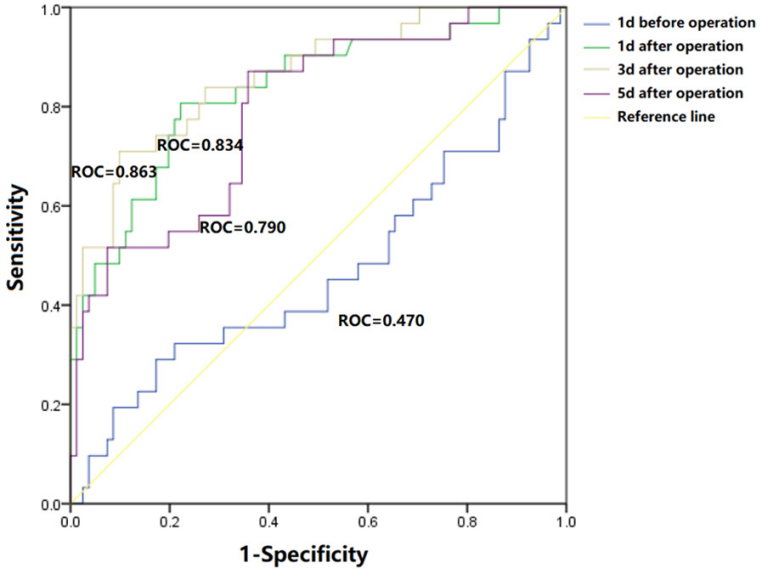

Figure 2. ROC curve analysis of the predictive value of D-dimer for DVT.

with D-dimer (r=0.387, *P<0.05*), as shown in **Figure 3**.

Discussion

Lower extremity DVT is one of the frequent complications after total hip arthroplasty, most of which occurs in perioperative period. As lower extremity venous thrombosis has a high incidence and is hard to detect, misdiagnosis and missed-diagnosis often occur in clinical diagnosis [9]. Patients in acute stage of DVT is prone to thrombus shedding and fatal pulmonary embolism, which is exactly the key cause in sudden clinical death [10, 11]. If the treatment is delayed or proceeds with poor efficiency, it may evolve into post-thrombosis syndrome, affect the limb function of the patient long-term, and lead to the formation of venous ulcers [12, 13]. Since the pathogenesis of DVT has not yet been clarified, research on its molecular mechanism is of great value for the early diagnosis and prediction of DVT after total hip arthroplasty.

miRNA is an endogenous non-coding small RNA consisting of 20-25 nucleotides, and is also a crucial post-transcriptional regulatory factor in organisms. miRNA can be involved in various biologic processes such as embryonic development, cell differentiation, and apoptosis [14, 15]. Current studies have shown that miRNA expression in PBMCs of patients with various diseases such as myocardial infarction, Parkinson disease, tumor, cerebral infarction, and diabetes is different from that of healthy people. Therefore, the miRNA in PBMCs is expected to be a new biomarker for diagnosis, treatment, and prognosis for the disease [18]. As an important subtype of miRNA, mir-374a-5p is abnormally expressed in PBMCs of patients with DVT, and is likely to become a new target for treating DVT [19, 20].

In this study, we analyzed the predictive value of miR-374a-5p in lower extremity DVT after total hip arthroplasty of elderly patients. The research outcomes reflected that the relative expression level of miR-374a-5p in PBMCs of the two groups 1 d, 3 d, and 5 d postoperatively were higher than that 1 d before surgery, and the indexes of thrombosis-group-patients were

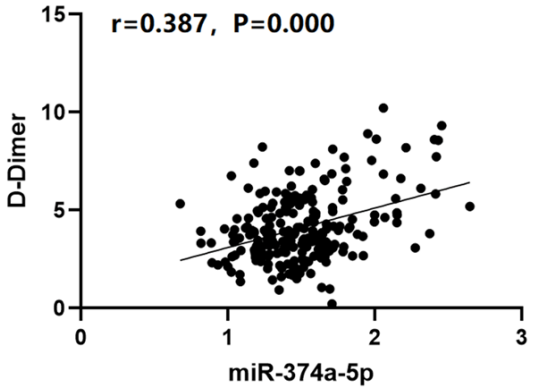

Figure 3. Analysis of correlation between miR-374a-5p and D-dimer.

remarkably higher than those in non-thrombosis-group at each time point. This result was consistent with those reported by scholars [21, 22], that the expression of miR-374a-5p in PBMCs of patients with total hip arthroplasty is increased, and this increase is more obvious in patients with DVT. The data suggest that miR-374a-5p may be involved in the occurrence of DVT, but the specific regulatory mechanism still needs to be further explored.

D-dimer is a specific degradation product of fibrin monomer hydrolyzed by fibrinolytic enzyme. When blood is hypercoagulable or fibrinolytic status, D-dimer can be specifically elevated, indicating the presence of coagulation and activation of the secondary fibrinolytic system in the body. At present, the detection of D-dimer has become a common method for predicting DVT [23, 24]. According to this research result, the relative expression of plasma D-dimer in the two groups 1 d, 3 d, and 5 d postoperatively was evidently higher than that 1 d before surgery, and the thrombosis-group-patients had higher relative expression of plasma D-dimer than non-thrombosis-group at each time point after surgery. This situation, which is consistent with other reportssuggests that D-dimer is increased in patients after surgery, and those with DVT have a more obvious increase of D-dimer [25, 26].

In order to further prove the effects of miR-374a-5p in the process of DVT, this study analyzed its correlation with D-dimer. The results showed that miR-374a-5p was positively correlated with D-dimer, suggesting that miR-374a-5p and D-dimer may interact with each other to promote the occurrence and progression of DVT. We analyzed the predictive value of miR-374a-5p by ROC curve method, and the area under the predictive curve of miR-374a-5p on 1 d pre-surgery, and 1 d, 3 d, and 5 d post-surgery for DVT were 0.435, 0.820, 0.974, and 0.782 respectively. This indicated that the postoperative miR-374a-5p has a high predictive value for DVT, among which the postoperative 3 d value is the most valuable one.

To conclude, miR-374a-5p is of greatly value in predicting DVT in elderly patients with total hip arthroplasty. Its expression level has a critically positive correlation with D-dimer degree, which is likely to be a biologic indicator for the early diagnosis and treatment of DVT.

Disclosure of conflict of interest

None.

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