Research Article

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Homocysteine is potential serological marker for predicting the risk of deep venous thrombosis of the lower extremities in patients received operation of lower limb fracture

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Abstract

Objective – The aim of the study is to investigate the correlations among serum homocysteine (Hcy), D-dimer, and the risk of developing deep venous thrombosis (DVT) of the lower extremities in patients who underwent operation for lower limb fracture.

Methods – Seventy-five cases who underwent operation for lower limb fracture were included and further divided into DVT group ($n = 26$) and control group ($n = 49$) based on post-DVT diagnostic criteria. The serum Hcy and D-dimer were examined 48 h after operation. The serum Hcy and D-dimer levels were compared between the two groups. The correlation between serum Hcy and D-dimer was investigated by the Pearson correlation test. The receiver-operating characteristic (ROC) curve was applied to evaluate the diagnostic performance of serum Hcy and D-dimer as serological markers for DVT.

Results – The serum Hcy concentrations were 11.96 ± 3.98 $\mu\text{mol/L}$ and 7.92 ± 3.27 $\mu\text{mol/L}$ for DVT and control groups, respectively, with statistical difference ($t = 4.72$, $P < 0.01$). The serum D-dimer in the DVT group was significantly higher than that of the control group (8.99 ± 4.50 vs 1.70 ± 2.11) $\mu\text{g/mL}$ with statistical difference ($t = 9.56$, $P < 0.01$). Line regression analysis indicated that serum Hcy was positively correlated with serum D-dimer concentration and can be demonstrated by the equation of $Y = 0.6651 \cdot X + 1.036$ for the DVT group. Using serum Hcy as

the biomarker for predicting DVT, the prediction sensitivity and specificity were 76.92 and 71.44%, respectively, with the AUC of 0.7804 under the cut-point of 9.54 $\mu\text{mol/L}$. For serum D-dimer, the prediction sensitivity and specificity were 96.15 and 73.47%, respectively, with the area under the ROC (AUC) of 0.9455 under the cut-point of 1.66 $\mu\text{g/mL}$.

Conclusion – Serum Hcy was significantly elevated in DTV patients, and hence, it can be applied as a serological marker for DVT prediction in patients who underwent operation for lower limb fracture. However, the DVT prediction performance of serum Hcy was inferior to D-dimer especially for diagnostic sensitivity.

Keywords: homocysteine, deep venous thrombosis, diagnosis, D-dimer

1 Introduction

About 1.5 of 1,000 adults suffer from first venous thrombosis in the developed countries in 1 year [1]. Deep venous thrombosis (DVT) occurs in the upper extremities in about 4–10% of cases [2], with an incidence of 0.4–1.0 people out of 10,000 in a year [3,4]. However, the DVT of lower extremities is more common than that of upper extremities especially for patients who underwent operation for the lower extremities or pelvic surgeries. It was reported that the DVT was the most developed complication for patients who underwent operation for lower limb fracture [5]. The risk for pulmonary embolism was significantly increased in patients with DVT, and the death risk was subsequent obviously elevated. Therefore, how to decrease the DVT incidence or how to screen high risk cases for the DVT was important for management of peri-operative complications in patients who underwent operation for lower limb fracture.

The previous studies [4,6,7] have indicated that serum markers such as D-dimer and tissue-type plasminogen

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activator were elevated in cases of DVT and could be applied for DVT diagnosis. Homocysteine (Hcy) is a homologue of the amino acid cysteine, which differ by an additional methylene bridge ($-\text{CH}_2-$). Under physiological conditions, Hcy can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins. The condition of the elevated serum Hcy (generally more than $15 \mu\text{mol/L}$) is called as hyperhomocysteinemia, which was associated with a wide range of diseases such as thrombosis [8], kidney disease [9], heart diseases [10], cancer [11], and neuropsychiatric illness [12]. In this study, we investigated the serum Hcy level in DVT and control groups in patients who underwent surgery for lower limb fracture and evaluated its diagnostic or predicting performance.

2 Material and methods

2.1 Patients

Seventy-five patients who underwent operation for lower limb fracture were included and further divided into DVT group ($n = 26$) and control group ($n = 49$) based on post-DVT diagnostic criteria. The inclusion criteria of this study were as follows: (1) patients older than 18 years, (2) patients with lower limb fracture, (3) patients underwent operation, (4) serum Hcy and D-dimer levels were examined 48 h after operation, and (5) DVT diagnosis was confirmed by lower extremity vascular ultrasound. Exclusion criteria of this study were as follows: (1) bone

fracture other lower extremity, (2) malignant carcinoma, (3) pregnant woman, (4) hematological diseases, and (5) combined with vascular injury. The general characteristics of the included cases are presented in Table 1.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

Informed consent: Informed consent has been obtained from all individuals included in this study.

2.2 Serum Hcy and D-dimer detection

Four milliliters of peripheral venous blood was collected from patients of the two groups at 48 h after operation. The peripheral venous blood was further divided into two equal parts (each 2 mL). One part was placed in the tube containing sodium citrate, and the other was placed in the tube containing EDTA-K2. The blood was centrifuged with the speed of 3,000 rpm for 15 min. After centrifugation, plasma samples were taken as soon as possible for D-dimer and Hcy detection. Latex agglutinate immunoturbidimetry and chemiluminescence immunoassay were performed for D-dimer and Hcy detection, respectively, in the blood containing sodium citrate and EDTA-K2.

2.3 Ultrasound examination

Siemens ACUSON $\times 700$ color Doppler ultrasound diagnostic instrument was used for DVT examination with the probe frequency of 5–8 MHz. The bilateral lower limbs of the patients were well exposed, and the fibular vein, popliteal vein, femoral vein, anterior tibial vein, and posterior tibial vein were scanned by ultrasound. Longitudinal and transverse sections were carried out to show the wall and lumen of venous vessels. The existence and nature of thrombosis were observed. Meanwhile, compression scanning was performed to evaluate the vascular filling (Figure 1).

2.4 Statistical analysis

STATA11.0 statistical software (<http://www.stata.com>) was used for the data analysis. The measurement data were expressed as $\bar{x} \pm s$, and the comparison between

Table 1: The general characteristics of the DVT and controls cases

Groups	DVT ($n = 26$)	Control ($n = 49$)	t/χ^2	P
Age (year), n	59.2 ± 12.3	56.8 ± 14.8	0.71	0.48
Gender			0.32	0.57
Male	12 (46.2%)	26 (53.1%)		
Female	14 (53.8%)	23 (46.9%)		
BMI			0.15	0.69
<24	18 (69.2%)	36 (73.5%)		
≥ 24	8 (30.8%)	13 (26.5%)		
Fracture type			1.83	0.18
Hip fracture	4 (15.4%)	5 (10.2%)		
Fracture of femoral shaft	2 (7.7%)	8 (16.3%)		
Distal femur fracture	3 (11.5%)	10 (20.4%)		
Patellar fractures	2 (7.7%)	8 (16.3%)		
Proximal fracture of tibia and fibula	5 (19.2%)	9 (18.4%)		
Distal tibia and fibula	6 (23.1%)	6 (12.2%)		
Ankle fracture	4 (15.4%)	3 (6.1%)

Figure 1: Doppler ultrasound in detection DVT: (a) hypoechoic filling could be seen in the left common femoral vein and the proximal femoral vein; (b) a dilated intramuscular vein with the widest inner diameter of about 0.5 cm can be seen in the right leg, and a low echo filling can be observed in the lumen.

groups was made by the Student *t*-test of the sample mean. The enumeration data were expressed as relative number, and the chi-square test was used for comparing between groups. DVT prediction sensitivity and specificity were calculated by the following equations: sensitivity = true positive/(true positive + false negative), and specificity = true negative/(true negative + false positive).

The area under the receiver-operating characteristic (ROC) curve was applied for evaluation of feasibility of serum Hcy and D-dimer as biomarkers for DVT prediction. Two-tailed $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Serum Hcy and D-dimer level between DVT and control groups

The serum Hcy concentrations were $11.96 \pm 3.98 \mu\text{mol/L}$ and $7.92 \pm 3.27 \mu\text{mol/L}$ for DVT and control groups, respectively, with statistical difference ($t = 4.72$, $P < 0.01$; Figure 2a). The serum D-dimer in the DVT group was significantly higher than that of the control group (8.99 ± 4.50 vs 1.70 ± 2.11) $\mu\text{g/mL}$ with statistical difference ($t = 9.56$, $P < 0.01$; Figure 2b).

3.2 Correlation between Hcy and D-dimer level in DVT group

Line regression analysis indicated that the serum Hcy was positively correlated with the serum D-dimer concentration and can be demonstrated by the equation of $Y = 0.6651X + 1.036$ for the DVT group (Figure 3).

3.3 Diagnostic performance of serum Hcy and D-dimer as serological markers for DVT

By using the serum Hcy as the biomarker for predicting the DVT, the prediction sensitivity and specificity were

Figure 2: The scatter plot of serum Hcy and D-dimer level between DVT and control groups: (a) serum level of Hcy in the DVT group was significantly higher than that of controls group and (b) serum level of D-dimer in the DVT group was significantly higher than that of control groups.

Figure 3: Correlation analysis between serum Hcy and D-dimer in the DVT group.

76.92 and 71.44%, respectively, with the AUC of 0.7804 under the cut-point of 9.54 $\mu\text{mol/L}$ (Figure 4a). For serum D-dimer, the prediction sensitivity and specificity were 96.15 and 73.47%, respectively, with the AUC of 0.9455 under the cut-point of 1.66 $\mu\text{g/mL}$ (Figure 4b). The summary of the serum Hcy and D-dimer as the biomarker for DVT prediction was presented in Table 2.

4 Discussion

In this study, we included 75 patients who underwent operation for lower limb fracture and detected the serum Hcy and D-dimer levels. We found that the serum Hcy and D-dimer levels in the DVT group was obviously elevated compared with that in non-DVT controls, with statistical difference in 48 h after operation. Applying the D-dimer and Hcy as serological markers for DVT prediction, the diagnostic sensitivity, specificity, and AUC

Table 2: The prediction efficacy of serum Hcy and D-dimer for DVT

Reference	Hcy	D-Dimer
Sensitivity	76.92%	96.15%
Specificity	71.44%	73.47%
Positive likelihood ratio	2.6923	3.6243
Negative likelihood ratio	0.3231	0.0524
AUC	0.7804	0.9455
Cut-point value	9.54	1.66
P-value	<0.01	<0.01

were 96.15%, 73.47%, and 0.9455 for serum D-dimer and 76.92%, 71.44%, and 0.7804 for serum Hcy, respectively. We also identified that the serum Hcy was positively correlated with the serum D-dimer concentration and can be demonstrated by the equation of $Y = 0.6651 \cdot X + 1.036$. These findings indicated that the serum Hcy was significantly elevated in DTV patients, and hence, serum Hcy can be applied as a serological marker for DVT prediction in patients who underwent surgery for lower limb fracture. However, the DVT prediction performance of serum Hcy was inferior to D-dimer especially for diagnostic sensitivity.

Serum D-dimer is an index, which has been widely used in clinical diagnosis, curative effect evaluation, and prognosis judgment of thrombotic diseases. D-Dimer is the fibrin degradation product, which can reflect the existence of hypercoagulable state and secondary hyperfibrinolysis in human body. It has been reported that the sensitivity of D-dimer in the diagnosis of venous embolic diseases is as high as 92–100%, but its specificity was relative low [13]. In this present study, we found that the prediction sensitivity and specificity were 96.15 and 73.47%, respectively, with the AUC of 0.9455 under the cut-point of 1.66 $\mu\text{g/mL}$ for serum D-dimer. The specificity was lower than the previous study [13]. This may be due

Figure 4: The ROC curve of serum Hcy and D-dimer as diagnostic reference for DVT: (a) ROC curve of serum Hcy in prediction of DVT and (b) ROC curve of serum D-dimer in prediction of DVT.

to the sample collection time points and DVT severity. Studies also identified that the level of serum D-dimer increased at the early stage of anticoagulant therapy or after thrombolysis and then decreased gradually, indicating that D-dimer may have important clinical significance in monitoring the curative effect and guiding the individualization of anticoagulant therapy [14,15].

Hcy is a sulfhydryl amino acid, which is an important intermediate of methionine metabolism [16]. In recent years, studies indicate that the increase of the plasma Hcy level was positively correlated with deep vein thrombosis [17,18]. The increase of the serum Hcy level leads to vascular endothelial cell injury and dysfunction. Hcy can harm the vascular endothelial system and then activate platelets and the coagulation system, which are involved in the formation of venous thrombosis [19]. The increase of the Hcy level can directly or indirectly damage the function of platelet cells in blood vessels, resulting in the deposition of cholesterol and lipoproteins in the vascular wall and can inactivate the vasodilator nitric oxide (NO), thus playing a role in promoting atherosclerosis and thrombosis [20–23]. Therefore, hyperhomocysteinemia caused by abnormal Hcy metabolism is a risk factor for atherosclerosis and thrombosis. Tan et al. [24] evaluated the clinical significance of combined detection of homocysteine, von Willebrand factor, and D-dimer in patients with lower extremity DVT. They found that the plasma levels of Hcy and D-dimer in patients with DVT were significantly increased, which indicated that Hcy and D-dimer had important clinical value for early diagnosis of DVT. In this study, we found that the Hcy serum level was significantly elevated in DVT groups and can be applied as a serological marker for DVT predication with relative high prediction sensitivity and specificity, which was in accordance with the study by Tan et al.

However, the serum level of Hcy can be affected by vitamin B6, vitamin B9, and vitamin B12 supplementation. Therefore, the administration of aforementioned vitamins may be potential confounding factors that can affect the Hcy prediction performance of DVT.

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