

## **The effect of VEGF on deep venous thrombosis in the perioperative period of elderly fracture patients.**

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### **Abstract**

**Objective:** We compared the correlation between serum Vascular Endothelial Growth Factor (VEGF) with D-Dimer, fibrinogen and thromboelastogram in order to investigate the effects of VEGF on deep venous thrombosis in the perioperative period of elderly fracture patients.

**Methods:** 68 elderly fracture patients that had undergone surgery were divided into two groups according to whether they were diagnosed with deep venous thrombosis in the perioperative period or not. We used the ELISA assay to detect the VEGF, D-Dimer and fibrinogen levels before the operation and 1 d, 5 d and 10 d post-operation. Thromboelastogram of patients before operation and after operation were recorded and further statistical analysis was conducted.

**Results:** The serum VEGF levels in the thrombosis group and non-thrombosis group both increased first and then decreased post-operation. After the operation at each of the different time points, the thrombosis group showed significant higher levels of serum VEGF compared to the non-thrombosis group ( $p<0.05$ ). Serum VEGF levels in the thrombosis group increased significantly after operation and its peak level was higher than that of the non-thrombosis group. In addition, VEGF levels in the thrombosis group after the operation were positively correlated with the D-Dimer and fibrinogen content ( $R^2=0.892$ ,  $p<0.05$ ;  $R^2=0.921$ ,  $p<0.05$ ). However, for non-thrombosis group, the positive correlations between VEGF levels and the content of D-Dimer and fibrinogen were relatively weak ( $R^2=0.634$ ,  $p<0.05$ ;  $R^2=0.611$ ,  $p<0.05$ ).

**Conclusion:** VEGF levels have a certain relationship with the D-Dimer and fibrinogen content in the formation of deep venous thrombosis in elderly patients with fracture. This relationship indicates the changing of blood coagulation state and fibrinolysis process in the formation of deep venous thrombosis. The up-regulated expression of VEGF, to some extent, will guide the treatment process and prognosis of deep venous thrombosis.

**Keywords:** VEGF, D-dimer, Deep venous thrombosis, Fibrinogen.

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### **Introduction**

68 cases of elderly fracture patients (>65 y old), who were treated in the orthopedic clinic of our hospital from January 2013 to December 2015, were recruited for our study. 41 cases were male and 27 cases were female; their ages ranged from 65 to 84 with an average of (72.03 ± 4.36). There were 18 femoral neck fractures cases, 16 pelvic fractures cases, 12 femoral shaft fractures cases, 12 tibial plateau fracture cases and 10 ankle joint fracture cases. All patients were operated on and 29 of them experienced Deep Venous Thrombosis (DVT) during perioperative period. This study was approved by the ethics committee of our hospital, and all the patients signed informed consent.

### **Groups and Testing Method**

68 cases were divided into the thrombosis group (29 cases) and non-thrombosis group (39 cases). We extracted 5 ml of the upper limb venous blood through the ulnar vein in the morning after having fasted all night for different times: before operation (T1), after operation for 1 day (T2), 5 days (T3) and 10 days (T4). We detected VEGF, D-Dimer and fibrinogen using the ELISA test and thromboelastogram test was performed for all patients before and post-operation.

### **Statistical analysis**

SPSS20.0 (IBM US) statistical software was used in our research data statistical analysis. The measurement data was evaluated by using mean ± standard deviation. Mono-factor

analysis of variance was used for analyzing the measurement difference between different groups. If the difference was statistically significant, a further LSD test proceeded between the two different groups. Correlations between VEGF, D-Dimer and fibrinogen content were analysed by Pearson correlation coefficient analysis,  $R^2$  represents the strength of the correlation.  $p < 0.05$  is regarded as statistically significant.

## Results

### *Comparison of general conditions*

In the thrombosis group, there were 16 cases of males and 13 cases of females with an average age of  $73.12 \pm 4.62$  y old, an average weight of  $79.48 \pm 8.76$  Kg and an average BMI of  $25.59 \pm 2.28$ . In the non-thrombosis group, there were 25 cases of males and 14 cases of females with an average of age  $71.65 \pm 4.14$  y old, an average weight of  $75.32 \pm 7.43$  Kg and an average BMI of  $24.24 \pm 2.19$ . Comparison of the general conditions showed that there were evident differences in weight and BMI between these two groups ( $p < 0.05$ ). It indicated that the heavier group and higher BMI group were more likely have DVT; there were no significant differences in gender and age between these two groups ( $p > 0.05$ ). Results are shown in Table 1.

### *Serum VEGF levels*

We compared the serum VEGF levels of the thrombosis group and the non-thrombosis group before operation; differences were not statistically significant ( $p > 0.05$ ). After the operation, serum VEGF levels in both groups showed an increase at first, followed by a decrease, which indicates that the operation can cause an increase in serum VEGF levels. The thrombosis group showed higher VEGF levels compared to the non-thrombosis group at different time points after the operation; differences were statistically significant ( $p < 0.05$ ). Serum VEGF levels in the thrombosis group increased significantly and its peak levels were significantly higher than the non-thrombosis group ( $p < 0.05$ ), which indicates that the formation of thrombus was closely related to the increase in VEGF level (Table 2).

### *Serum D-Dimer content*

We compared the serum D-Dimer content of the thrombosis and non-thrombosis group before the operation; differences were not statistically significant ( $p > 0.05$ ). The serum D-Dimer content in the thrombosis and non-thrombosis group increased at the beginning, followed by a decrease which indicates that

the operation can cause an increase in serum D-Dimer content. The thrombosis group showed a higher serum D-Dimer content compared to the non-thrombosis group after treating for different days; differences were statistically significant ( $p < 0.05$ ). The serum D-Dimer content in the thrombosis group increased significantly and its peak level was significantly higher than the non-thrombosis group ( $p < 0.05$ ), which indicates that the formation of thrombosis was closely related to the increase of D-Dimer content (Table 3).

The positive correlation between VEGF levels and D-Dimer content in the thrombosis group at T2 was strong ( $R^2 = 0.892$ ,  $p < 0.05$ ). For the non-thrombosis group, this positive correlation was relatively weak ( $R^2 = 0.634$ ,  $p < 0.05$ ).

### *Fibrinogen content in peripheral venous serum*

The comparison of fibrinogen content between the thrombosis and non-thrombosis group showed that the difference was not statistically significant before operation ( $p > 0.05$ ). Fibrinogen content in both groups showed an increase at the beginning, followed by a decrease post-operation, which indicates that operation can cause an increase in serum fibrinogen content. We compared the fibrinogen content between two groups at different time points after operation. Thrombosis group had higher rates than the non-thrombosis group; difference was statistically significant ( $p < 0.05$ ). The thrombosis group experienced a more significant increase of fibrinogen content and the peak level of fibrinogen content was significantly higher than the non-thrombosis group ( $p < 0.05$ ), which indicates that the formation of thrombosis was closely related to the increase of fibrinogen content (Table 4).

The positive correlation between VEGF levels and fibrinogen content in the thrombosis group at T2 was strong ( $R^2 = 0.921$ ,  $p < 0.05$ ). For the non-thrombosis group, this positive correlation was relatively weak ( $R^2 = 0.611$ ,  $p < 0.05$ ).

### *The thromboelastogram results comparison between before and after operation*

Before the operation, 7 cases in thrombosis group (24.14%) and 10 cases in non-thrombosis group (25.64%) showed low blood coagulation; the difference was not statistically significant ( $p > 0.05$ ). After the operation, 4 cases in thrombosis group (13.79%) and 16 cases in non-thrombosis group (41.03%) experienced a reduction of blood coagulability, and differences were statistically significant ( $p < 0.05$ ).

**Table 1.** Comparison of general conditions.

Groups	N	Gender		Average age (year)	Weight (Kg)	Weight Index
		Male	Female			
Thrombosis	29	16	13	$73.12 \pm 4.62^a$	$79.48 \pm 8.76^a$	$25.59 \pm 2.28^a$
Non-thrombosis	39	25	14	$71.65 \pm 4.14^a$	$75.32 \pm 7.43^a$	$24.24 \pm 2.19^a$

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$\chi^2$ or t value	0.554	1.3781 <sup>b</sup>	2.1151 <sup>b</sup>	2.4704 <sup>b</sup>
p value	0.4567	0.1728	0.0382	0.0161

Note: a data is expressed as  $\bar{x} \pm s$ , b is t value.

Table 2. VEGF level ( $\bar{x} \pm s$ , pg/ml) in peripheral venous serum.

Group	N	T1	T2	T3	T4
Thrombosis	29	345.5 ± 44.7	499.3 ± 77.5	588.2 ± 97.6	395.8 ± 66.7
Non-thrombosis	39	363.1 ± 51.3	441.6 ± 83.2	493.5 ± 84.3	364.3 ± 59.3
T value		1.4766	2.9112	4.2826	2.0539
p value		0.1445	0.0049	0.0001	0.0439

Table 3. D-Dimer content in peripheral venous serum ( $\bar{x} \pm s$ , ng/ml).

Group	N	T1	T2	T3	T4
Thrombosis	29	65.5 ± 11.2	196.3 ± 57.5	261.2 ± 77.4	88.5 ± 14.7

Non-thrombosis	39	63.1 ± 10.3	159.4 ± 44.2	198.5 ± 61.2	69.2 ± 12.1
T value		0.9155	2.9934	3.7307	5.9335
p value		0.3633	0.0039	0.0004	0

Table 4. Fibrinogen content in peripheral venous serum ( $\bar{x} \pm s$ , mg/dL).

Group	N	T1	T2	T3	T4
Thrombosis	29	125.5 ± 21.2	172.5 ± 36.2	219.5 ± 51.4	181.5 ± 44.6
Non- thrombosis	39	128.4 ± 25.3	153.4 ± 34.6	164.5 ± 41.1	139.2 ± 35.5
T value		0.5001	2.2074	4.9024	4.3545
p value		0.6186	0.0308	0	0

Table 5. Thromboelastogram results comparison between before and after operation.

Group	Cases	Thromboelastogram low blood coagulation patients (n)	
		Before operation	After operation
Thrombosis	29	7	4
Non- thrombosis	39	10	16
$\chi^2$ value		0.02	5.9412
p value		0.8874	0.0148

## Discussion

Deep venous thrombosis is a very common complication of orthopedic patients who are on bed rest. Its clinical manifestations include unilateral or bilateral lower limb swelling, post-thrombotic syndrome and lethal pulmonary embolism which can severely affect a patients' health [1-5]. It has been reported that the incidence of deep venous thrombosis is approximately 1-2% worldwide [6-8]. With regards to China, despite the sparsity of multicenter epidemiology investigation data available for deep venous thrombosis, it still be discerned that its incidence is high according to the analysis of literature and clinical data [7,8]. Deep venous thrombosis refers to the abnormal coagulation of blood in the lower limbs deep vein

system which will block the venous duct and cause acute obstruction of lower limbs venous backflow and increase of internal pressure; it will also damage the venous valve and cause a series of symptoms and signs [9,10]. German physiologist Virchow pointed that the main causes of deep venous thrombosis are slow venous blood flow, hypercoagulable state and venous wall damage; the first two are the main reasons. Any disease which can cause the three problems mentioned above will be the risk factors for deep venous thrombosis [11].

VEGF is a specific vascular permeability factor and chemotactic factor of vascular endothelial cells [12]. During the embryonic growth and development stage, the formation of

the embryonic vascular system is dependent on the correct expression of VEGF. After birth, VEGF still plays an important role in the formation of blood vessels. Endothelial progenitor cells can migrate to peripheral blood when signalled by exogenous VEGF. VEGF can improve the proliferation, migration and chemokines of endothelial progenitor cells, which can also promote angiogenesis. It has been reported that the number of endothelial progenitor cells in the recycling of peripheral blood increased significantly in a VEGF-rich microenvironment. Also a hypoxic microenvironment can further improve the proliferation and differentiation of endothelial progenitor cells and finally enhance the effects of endothelial progenitor cells on improving angiogenesis. After the formation of thrombosis, due to the blood flow stasis and hypoxic microenvironment, VEGF and BFGF content inside of the emboli increase significantly. Therefore, the thrombolysis and organization speed increases [13]. The possible antithrombotic mechanism of VEGF is as follows: 1. act as an endogenous regulator of body parts, not only participate in the maintenance of the integrity of vascular endothelial cells, but also in the maintenance of normal physiological functions to prevent the occurrence of endogenous and exogenous coagulation. 2. VEGF can increase the production of NO and PGI [14]. NO can activate Soluble Guanylate Cyclase (SGC) to increase cGMP content inside of the cell, which can finally cause platelet aggregation and block property decrease. 3. VEGF can also promote the expression and activity of serine protease, fibrinoclastase, urokinase and tissue-plasminogen. These enzymes can cause the conversion of plasminogen to plasmin which will finally improve the thrombolysis [15].

It has been reported that D-Dimer, fibrinogen and inflammatory cellular factors in the peripheral blood are closely related to the formation of deep venous thrombosis [16]. After the activation of the fibrinolytic system of the body, fibrinogen will convert to a fibrin monomer to crosslink with the factor XIII. D-Dimer is the product of the fibrinolytic enzyme hydrolysis process. The accumulation of fibrinogen in plasma can enhance fibrinolytic activity and also increase D-Dimer content. Therefore, D-Dimer can specifically induce the hypercoagulable state of blood and hyperfunction of the fibrinolysis process at the molecular level [17,18]. D-Dimer index can increase several times during the formation of deep venous thrombosis [19,20]. The clinical way to detect D-Dimer and fibrinogen is easy and efficient, which is of great significance in the diagnosis of deep venous thrombosis and evaluation of the prognosis.

Our study has found that VEGF levels in both these two groups showed an increase at first and followed by a decrease, which indicates that the angiogenesis process after operative injury can cause an increase of serum VEGF levels. The thrombosis group showed higher VEGF levels compared to the non-thrombosis group after the operation for different days; differences were statistically significant ( $p < 0.05$ ). Serum VEGF levels in the thrombosis group increased significantly and its peak level was significantly higher than the non-thrombosis group which indicates that the formation of deep venous thrombosis can stimulate the expression of VEGF to

improve the thrombolysis process. Meanwhile, VEGF levels in the thrombosis group after the operation days were positively correlated with the D-Dimer and fibrinogen content ( $R^2=0.892$ ,  $p < 0.05$ ;  $R^2=0.921$ ,  $p < 0.05$ ). However, for the non-thrombosis group, the correlation between VEGF levels and the content of D-Dimer and fibrinogen was relatively weak ( $R^2=0.634$ ,  $p < 0.05$ ;  $R^2=0.611$ ,  $p < 0.05$ ). This indicated that fractures trauma and operations can stimulate the expression of VEGF to improve neovascularization and repair the trauma. In the meantime, the formation of deep venous thrombosis can further increase the expression of VEGF in order to aid the thrombolysis process.

Moreover, thromboelastogram can fully reflect the shear stress changing with time in the dynamic clotting process and precisely describe the clotting mechanism. Our study has found that the amount of patients with lower blood coagulability in the thrombosis group is significantly smaller than that in non-thrombosis group ( $p < 0.05$ ). The dynamic monitoring of the coagulation process demonstrated that the blood coagulability of patients in the thrombosis group was in a rising state.

In conclusion, VEGF level was positively correlated with D-Dimer and fibrinogen content in the formation of deep venous thrombosis of elderly fracture patients. This correlation indicates the changing of blood coagulation state and the fibrinolysis process in the formation of deep venous thrombosis. The up-regulated expression of VEGF to some extent will guide the treatment process and prognosis of the deep venous thrombosis.

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