The effects of VEGF on deep venous thrombosis in the perioperative period of elderly fracture patients

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Abstract: Deep venous thrombosis is a very common complication of orthopedic patients on bed rest. In the present study, comparison of the correlation between serum vascular endothelial growth factor (VEGF) with D-Dimer, fibrinogen and thrombo elastogram was done. 68 elderly fractured 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. ELISA assays was carried out to detect the VEGF, D-Dimer and fibrinogen levels prior to operation and post-operation on 1st, 5th& 10th day. SPSS20.0 (IBM US) statistical software was used in the research for statistical data analysis. The measured data was evaluated by using mean ± standard deviation. The present study has found that VEGF levels in both these two groups showed an increase at first followed by a decrease, which indicated 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; difference was statistically significant (P<0.05). In addition, VEGF levels in the thrombosis group after the operation were closely related to the D-Dimer and fibrinogen content. However, for non-thrombosis group, the relationship between VEGF levels and the content of D-Dimer and fibrinogen was weak.

Keywords: VEGF, D-Dimer, deep venous thrombosis, fibrinogen.

INTRODUCTION

Deep venous thrombosis of elderly fracture patients is a common disease and encompasses multiple diseases which is mainly caused by slow venous blood flow, a hypercoagulable state and venous wall damage (Brummel *et al*., 2005; Stawicki *et al*., 2005). Fibrinogen is one of the most important coagulation factors made by the body itself and it plays an important role in the generation of coagulation and thrombus. The fibrinolytic enzyme is generated after the fibrinolytic system has been activated. D-Dimer is a specific product that is produced in the hydrolysis process of fibrinolytic enzymes and it reflects the progress of venous thrombus generation very well (Wells *et al*., 2003). Studies have shown that vascular endothelial growth factor (VEGF) is closely related to the vascular endothelial system and plays an important role in the formation of deep venous thrombosis (Bozoglu *et al*., 2005; Jacobson, 2005). In the thrombus organization process, an up-regulated expression of VEGF can improve the thrombus organization and recanalization. Studies have also reported that a down-regulated expression of VEGF has been found in the early stage of thrombosis, and the formation or thrombus can be affected by regulating the synthesis and release of NO. The present research mainly focuses on comparing the correlation between serum vascular endothelial growth factor (VEGF) with D-Dimer, fibrinogen and thrombo elastogram in order to investigate the effects of VEGF on deep venous thrombosis in the perioperative period of elderly fracture patients.

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MATERIALS AND METHODS

68 cases of elderly fracture patients (>65 years old), that were treated in the orthopedic clinic of First Affiliated Hospital of PLA General hospital, China 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 age of (72.03±4.36 years). 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 the perioperation**.** 68 cases were divided into the thrombosis group (29 cases) and non-thrombosis group (39 cases). 5mL of the upper limb venous blood was extracted through the ulnar vein in the morning after having fasted all night for different times: before operation (T1), after operation for 1st day (T2), 5th day (T3) and 10th day (T4). VEGF, D-Dimer and fibrinogen was detected, using the ELISA test and proceeded the thrombo elastogram test for all patients before and post-operation.

Ethical approval

Ethical approval was taken by Linzi District People's Hospital through No. 2017081123.

STATISTICAL ANALYSIS

SPSS (V: 20.0 IBM US) statistical software was used in the study for statistical data analysis. The measured 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 preceded between the two different groups. Analysis of the correlation between VEGF, D-Dimer and fibrinogen content was carried out. R^2 represents the strength of the correlation whereas $P<0.05$ is regarded as statistically significant.

RESULTS

Comparison of general conditions

The thrombosis group included 16 cases of male and 13 female cases, which were an average of 73.12±4.62 years old with average weights of 79.48±8.76 Kg and had an average BMI of 25.59±2.28. The non-thrombosis group comprised of 25 cases of male and 14 female cases which were an average of 71.65±4.14 years old, weighed an average of 75.32±7.43 Kg and had an average BMI of 24.24±2.19. Comparison of the general condition showed that there was an evident difference between weight and BMI in these two groups ($P<0.05$). It indicated that the heavier group and higher BMI group was more likely to have DVT. However, there were no significant differences between the gender and age of these two groups ($P>0.05$). Results are shown in table 1.

Serum VEGF levels

Comparison of the serum VEGF levels of the thrombosis group and the non-thrombosis group before operation showed statistically significant differences ($P<0.05$). After the operation, serum VEGF levels in both groups showed an increase at first, followed by a decrease, which indicated that the operation might cause an increase in 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 levels were significantly higher than the non-thrombosis group, which indicated that the formation of thrombus was closely related to the increase in VEGF level. The results are depicted in table 2.

Serum D-Dimer content

Serum D-Dimer content of the thrombosis and non-thrombosis group before the operation was compared; difference was statistically significant ($P<0.05$). The serum D-Dimer content in the thrombosis and non-thrombosis group first increased, followed by a decrease which indicated that the operation could 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; difference was 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, which indicated that the formation of thrombosis was closely related to the increase of D-Dimer content (table 3).

The relationship between VEGF levels and D-Dimer content in the thrombosis group at the time of T2 and T4 was strong and the Pearson factor was 0.681, $R^2=0.892$

Table 1: Comparison of general conditions

Groups	*n*	Gender		Average Age (year)	Weight (Kg)	Weight Index
		Male	Female			
Thrombosis	29	16	13	73.12±4.62[a]	79.48±8.76[a]	25.59±2.28[a]
Non-thrombosis	39	25	14	71.65±4.14[a]	75.32±7.43[a]	24.24±2.19[a]
χ^2 or t value		0.5540		1.3781[b]	2.1151[b]	2.4704[b]
P value		0.4567		0.1728	0.0382	0.0161

Note: a data is expressed as x±s, b is t-value.

Table 2: VEGF level（x±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 (x±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	0.0000

Table 4: Fibrinogen content in peripheral venous serum (x±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	0.0000	0.0000

Table 5: Thrombo elastogram results comparison between before and after operation

Group	Cases	Thrombo elastogram low blood coagulation patients (n)	
		Before operation	After operation
Thrombosis	29	7	4
Non- thrombosis	39	10	16
χ^2 value		0.0200	5.9412
P value		0.8874	0.0148

(*P*<0.05). For the non- thrombosis group, this relationship was weak and the Pearson factor was 0.451, 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 (*P*>0.05). Fibrinogen content in both groups showed an increase first followed by a decrease post-operation, which indicated that operation can cause an increase in serum fibrinogen content. Comparison was done for the fibrinogen content between two groups post-operation for different days. The, thrombosis group had higher rates than the non-thrombosis group; difference was statistically significant. 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, which indicated that the formation of thrombosis was closely related to the increase of fibrinogen content (table 4).

The relationship between VEGF levels and fibrinogen content in the thrombosis group at the time of T2 and T4 was strong and the Pearson factor was 0.742, R^2=0.921 (*P*<0.05). For the non-thrombosis group, this relationship was weak and the Pearson factor was 0.429, R^2=0.611 (*P*<0.05).

The thrombo elastogram results comparison; 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; difference was statistically significant (*P*<0.05). Results are shown in table 5.

DISCUSSION

Deep venous thrombosis is a very common complication of orthopedic patients 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 patient's health. It has been reported that the incidence of deep venous thrombosis is approximately 1-2% worldwide (Hoaglund, 2004; Leung *et al*., 2006). With regard 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 (Ko *et al*., 2003; Leung *et al*., 2006). Deep venous thrombosis refers to the abnormal coagulation of blood in the lower limbs deep venous 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 (Sillesen *et al*., 2005; Malone & Agutter, 2016). 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 (Cervantes & Rojas, 2005).VEGF is a specific vascular permeability factor and chemotactic factor of vascular endothelial cells (Trelinski *et al*., 2010). 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 signaled 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 (Cacciola *et al*., 2002). The possible antithrombotic mechanism of VEGF is as follows: (i) act as an endogenous regulator of body parts, not only in the participation of the maintenance of the integrity of vascular endothelial cells, but also to maintain the normal physiological functions to prevent the occurrence of endogenous and exogenous coagulation. (ii) VEGF can increase the production of NO and PGI (Wei *et al*., 2007). 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. (iii) VEGF can also promote the expression and activity of serine protease, fibrinoclase, urokinase and tissue-plasminogen. These enzymes can cause the conversion of plasminogen to plasmin which will finally improve the thrombolysis (Chun *et al*., 2001). 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 (Wuillemin *et al*., 2005). After the activation of the fibrinolytic system of the body, fibrinogen will convert to fibrin monomers which will 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 hyper function of the fibrinolysis process at the molecular level (Chan *et al*., 2010; Aguilar *et al*., 2002). D-Dimer index can increase several times during the formation of deep venous thrombosis (Bates *et al*., 2012; Schutgens *et al*., 2003). 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 indicated 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; difference was statistically significant. Serum VEGF levels in the thrombosis group increased significantly and its peak level was significantly higher than the non-thrombosis group which indicated that the formation of deep venous thrombosis can stimulate the expression of VEGF in order to improve the thrombolysis process. Meanwhile, VEGF levels in the thrombosis group after the operation for different days were closely related to the D-Dimer and fibrinogen content. However, for the non-thrombosis group, the relationship between VEGF levels and the content of D-Dimer and fibrinogen was weak. This indicated that fractures, trauma and operations can stimulate the expression of VEGF in order to improve neovascularization and to 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.

CONCLUSION

VEGF levels had a certain relationship with D-Dimer and fibrinogen content in the formation of deep venous thrombosis of elderly fracture patients. This relationship indicated 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. Moreover, thrombo elastogram can fully reflect the shear stress changing with time in the dynamic clotting process and precisely describe the clotting mechanism.

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