Clin. Lab. 2023;69:XXX-XXX

ORIGINAL ARTICLE

Correlation between Serum MMP-2/-16 Levels and Inflammation in Patients with Deep Vein Thrombosis

HongBin Gu [*], MingFei Li [*], HuaJie Xie, Fan Yang, ZhiHong Wang, Lei Sheng

[*] *These authors made equal contributions to this study*
Department of Vascular Surgery, Strategic Support Force Medical Center of PLA, Beijing City, China

SUMMARY

Background: **The aim of the study was to clarify the correlation between serum MMP-2/-16 and inflammation in patients with deep venous thrombosis (DVT).**
Methods: **Sixty DVT patients and 60 healthy people who underwent health examinations were collected. Serum MMP-2/-16, IL-6/-8, and TNF-α were determined by ELISA. MMP-2/-16 protein levels were detected by western blot, and IL-6/-8 and TNF-α by RT-qPCR. Correlation analysis was performed on MMP-2/-16xdd, IL-6/-8, and TNF-α in DVT patients.**
Results: **MMP-2/-16, IL-6/-8, and TNF-α in DVT patients after treatment were lower than before treatment. Serum IL-6/-8 and TNF-α levels in DVT patients were both positively correlated with MMP-2/-16 levels.**
Conclusions: **MMP-2/-16 and inflammatory factors are related to DVT development, and IL-6/-8 and TNF-α are positively correlated with MMP-2/-16.**
(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2023.230611)

Correspondence:
HongBin Gu
Department of Vascular Surgery
Strategic Support Force Medical Center of PLA
No. 9, Beijing City, 100101
China
Email: hongbingu@hotmail.com

AnXiang Bei Li
Chaoyang District
Beijing City, 100101
China

Manuscript accepted July 3, 2023

KEYWORDS

deep vein thrombosis, MMP-2, MMP-16, IL-6, IL-8, TNF-α

INTRODUCTION

Deep vein thrombosis (DVT) is a common peripheral vascular disease, which forms thrombosis in the deep vein of the human body [1]. DVT in the lower and upper extremities can cause post-thrombotic syndrome and pulmonary embolism, resulting in more than 15% mortality within the first 3 months after diagnosis [2]. Several studies have elucidated the key action of inflammatory response in DVT [3-6]. Inflammatory cytokines are bioactive peptides that not only act as signal transduction factors but as effector molecules [7]. Apart from directly causing endothelial cell damage, inflammatory factors can also activate the release of inflammatory factors by the coagulation system, further aggravating the inflammatory response and blood coagulation [8]. Matrix metalloproteinases (MMPs) are zinc-based enzymes capable of degrading extracellular matrix pro-

teins [9]. Under normal conditions, MMP expression is low, but the change of its expression and inhibitory factors can cause neovascularization, inflammation, tumor metastasis and other pathological reactions [10]. Inflammation and thrombosis are modified by MMPs and their inhibitors [11]. MMP-2 belongs to gelatinase, and increased expression level of the MMP-2 gene or activation enzyme activity may be involved in venous thromboembolism (VTE) [12]. Studies have suggested that MMP-2 is a key factor in lower limb venous diseases [13,14]. MMP-16 is a membrane-type metalloproteinase involved in cell proliferation, migration, and invasion [15] and activates proMMP-2 to modulate cell development [16,17].

To investigate the effects of MMP-2/-16 and inflammation-related factors on DVT, we selected 60 DVT patients and another 60 volunteers who underwent health examinations. Serum concentrations of MMP-2/-16, IL-6/-8, and TNF-α were detected. MMP-2/-16 protein, IL-6/-8, and TNF-α mRNA in peripheral blood mononuclear cells (PBMCs) of DVT patients were determined. The correlation between serum levels of MMP-2/-16, IL-6/-8, and TNF-α in DVT patients was analyzed, respectively.

MATERIALS AND METHODS

Patient population

Sixty patients diagnosed with DVT in Strategic Support Force Medical Center of PLA were selected as the DVT group. Inclusion criteria: 1) Patients who developed DVT within 7 days; 2) patients with similar conditions; 3) Patients with lower limb venous flow obstruction confirmed by anterograde lower limb deep vein angiography. Exclusion criteria: patients with pregnancy, infection, tumors, hereditary anticoagulant deficiency, vascular abnormalities, or diabetes. All patients had high muscle tone, swelling, and other clinical manifestations before treatment, and unilateral lower limb DVT was confirmed by color Doppler ultrasound or venography. Another 60 healthy people who received health examinations in Strategic Support Force Medical Center of PLA were selected as the normal control group. All of them were healthy adults without heart, liver, brain, kidney, infectious diseases, or diabetes. This study was approved by the Ethics Committee of Strategic Support Force Medical Center of PLA. An informed consent form signed by the patient or guardian was obtained from each participant.

Clinical treatment

Patients in the DVT group received 200,000 units of urokinase intravenously for 5 days from the first day of diagnosis, subcutaneous injection with low molecular weight heparin at 4,000 units, twice a day, and oral antiplatelet aggregation drug aspirin 100 mg, once a day.

Collection and testing of serum samples

In the morning before and 7 days after treatment, 5 mL of fasting venous blood was taken from DVT patients and normal controls. The blood was centrifuged at 4℃ and 4,000 g for 15 minutes, without anticoagulation treatment, thereby collecting supernatant which was kept at -80℃.

Serum levels of MMP-2/-16, IL-6/-8, and TNF-α were determined by ELISA.

PBMC isolation and culture

Before treatment and 7 days after treatment, 10 mL venous blood was extracted from DVT patients on an empty stomach, and PBMCs were separated by Ficoll-Paque PLUS. PBMCs were cultured in RPMI-1640 medium containing 10% serum at 37℃ and 5% CO_2 for 2 hours, and suspended cells were washed away. Adherent PBMCs were obtained for subsequent experiments.

Western blotting

PBMCs digested by trypsin were lysed with lysis buffer and centrifuged at 3,000 g at 4℃ for 10 minutes. The collected supernatant proteins were quantified using a BCA protein detection kit. Protein (30 μg) was processed by SDS-PAGE, transferred to PVDF membranes, sealed with a blocking buffer for 2 hours. The membranes were then incubated with MMP-2/-16, and GAPDH primary antibody (1:1,000) overnight at 4℃. After TBST treatments (3 times), the membrane was incubated with a secondary antibody (1:1,000) for 2 hours and then rinsed with TBST 3 times. The membrane was developed in an ECL solution (MilliporeSigma, USA) and scanned by a gel imager. Image J software was used for density analysis.

RT-qPCR

Total RNA was extracted from PBMCs digested by trypsin based on the TRIzol kit. RNAs with absorbance ratio (A260/A280) of 1.8 - 2.0 were reverse transcribed and cDNA was taken as a template. Table 1 presents the primer sequences, and the reaction conditions are as follows: pre-denaturation at 94℃ for 3 minutes, pre-denaturation at 95℃ for 1 minute, annealing at 50℃ for 50 seconds, extension at 72℃ for 1 minute, and amplification for 10 minutes, for a total of 40 cycles. Experimental results were analyzed by $2^{-\Delta\Delta Cq}$ method.

Statistical analysis

The SPSS 17.0 software was used for data processing. Measurement data were expressed as mean ± standard deviation, and comparative analysis was done using *t*-test. Pearson's correlation coefficient analysis was used to determine significance of comparison data, where $p < 0.05$ indicated a significant difference.

Table 1. Primers.

Genes	Sequences (5' - 3')
IL-6	Forward: CTGTTGTTGCTGTGGCTGAT
	Reverse: TCCGTCCACAAGCAATGAGT
IL-8	Forward: ATGACTTCCAAGCTGGCCGTGGCT
	Reverse: TCTCAGCCCTCTTCAAAAACTTCTC
TNF-α	Forward: TACTGAACTTCGGGGTGATTGGTCC
	Reverse: CAGCCTTGTCCCTTGAAGAGAACC
GAPDH	Forward: ATGGCACCGTCAAGGCTGAG
	Reverse: GCAGTGATGGCATGGACTGT

Table 2. General patient data.

Parameter	Control group (n = 60)	DVT group (n = 60)	p-value
Age (years)	51.48 ± 11.21	54.64 ± 12.35	0.145
Grade [n (male/female)]	33/27	32/28	0.855
Weight (kg, mean ± SD)	65.72 ± 12.49	62.65 ± 13.76	0.203

Table 3. Serum levels of MMP-2, MMP-16 and inflammatory cytokines in normal control group and DVT group.

Parameters	Control group	DVT group	p-value
MMP-2 (μmol/L)	0.917 ± 0.274	4.064 ± 0.525	< 0.001
MMP-16 (μmol/L)	0.633 ± 0.136	3.246 ± 0.563	< 0.001
IL-6 (μg/L)	0.095 ± 0.014	0.163 ± 0.048	< 0.001
IL-8 (μg/L)	0.353 ± 0.096	0.788 ± 0.162	< 0.001
TNF-α (μg/L)	16.12 ± 3.754	54.28 ± 11.39	< 0.001

Table 4. Serum levels of MMP-2, MMP-16 and inflammatory cytokines in DVT group before and after treatment.

Parameter	Before treatment	After treatment	p-value
MMP-2 (μmol/L)	4.064 ± 0.525	1.961 ± 0.448	< 0.001
MMP-16 (μmol/L)	3.246 ± 0.563	1.582 ± 0.373	< 0.001
IL-6 (μg/L)	0.163 ± 0.048	0.128 ± 0.026	< 0.001
IL-8 (μg/L)	0.788 ± 0.162	0.465 ± 0.154	< 0.001
TNF-α (μg/L)	54.28 ± 11.39	34.43 ± 8.962	< 0.001

RESULTS

General patient data

There were 32 males and 28 females in the DVT group, with an average age of 54.64 ± 12.35 years old. The normal control group included 33 males and 27 females, with an average age of 51.48 ± 11.21 years. Both groups suggested no significant difference in age, gender, and body weight (Table 2).

Table 5. Correlation analysis of serum MMP-2, MMP-16, and inflammatory factors in patients with DVT group.

	MMP-2		MMP-16	
	r	p-value	r	p-value
IL-6	0.654	< 0.001	0.5	< 0.001
IL-8	0.595	< 0.001	0.474	< 0.001
TNF-α	0.563	< 0.001	0.465	< 0.001

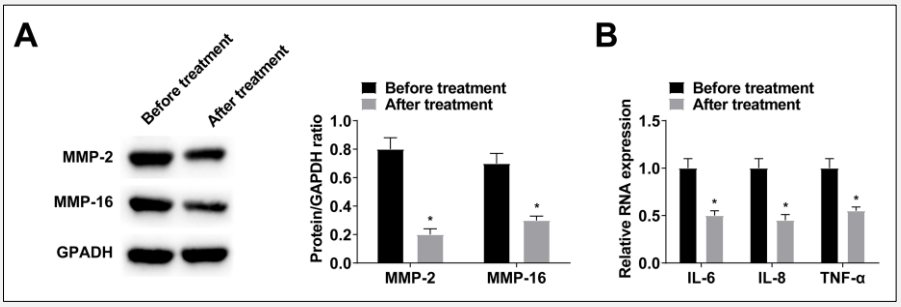

Figure 1. Levels of MMP-2/-16 and inflammatory factors in PBMCs of DVT patients.

A: Western blot analysis of MMP-2/-16 in PBMCs of DVT patients. B: RT-qPCR detection of IL-6/-8, and TNF-α in PBMCs of DVT patients; * - p < 0.05 vs. Before treatment.

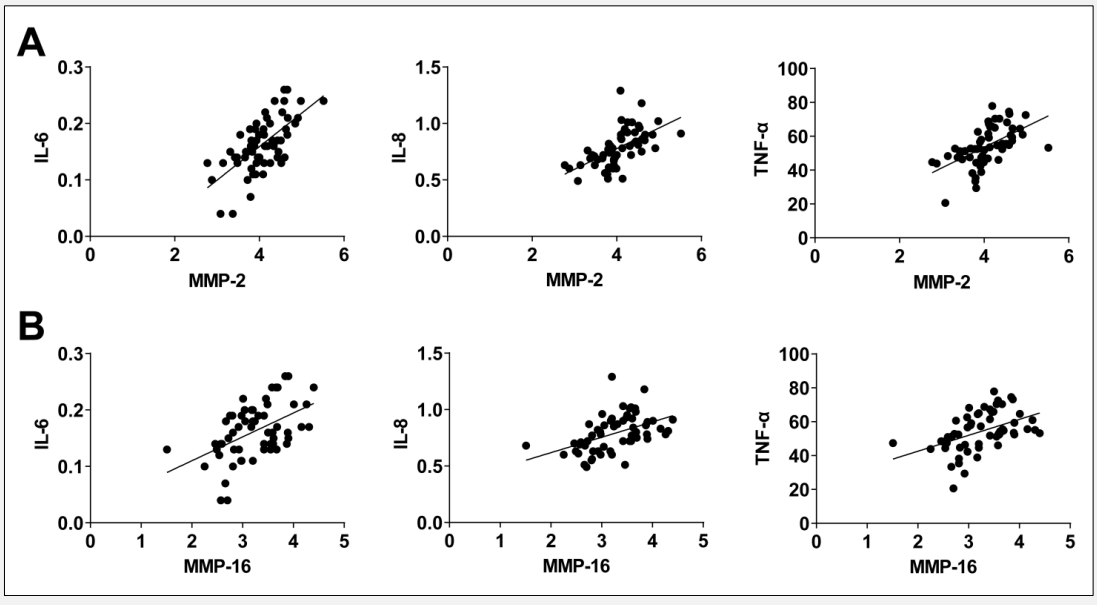

Figure 2. Correlation analysis of serum MMP-2/-16 and inflammatory factors in DVT patients.

A - B: Pearson's correlation analysis of MMP-2/-16, IL-6/-8, and TNF-α in DVT patients.

MMP-2/-16 and inflammatory cytokines before treatment

As measured by ELISA, DVT patients had higher MMP-2/-16, IL-6/-8, and TNF-α levels in the serum before treatment (Table 3).

Serum levels of MMP-2/-16 and inflammatory cytokines

ELISA results showed that after 7 days of treatment, serum MMP-2/-16, IL-6/-8, and TNF-α in DVT patients were significantly lower than before treatment (Table 4).

MMP-2/-16 and inflammatory cytokines in PBMCs of DVT patients

Western blot results showed that MMP-2/-16 proteins in PBMCs of DVT patients were decreased after treatment (Figure 1A). RT-qPCR results showed that IL-6/-8, and TNF-α levels in PBMCs of DVT patients decreased after treatment (Figure 1B).

Correlation analysis of serum MMP-2/-16 and inflammatory cytokines in DVT patients

Serum MMP-2/-16 and inflammatory factors in DVT patients were recorded, and Pearson correlation analysis determined that MMP-2/-16 in DVT patients were positively correlated with IL-6/-8, and TNF-α, respectively (Figure 2A, B, Table 5).

DISCUSSION

DVT occurs in deep veins, usually in the legs, but can also occur in the arms and other parts of the body [18]. It is estimated that the incidence of DVT is relatively high [19]. Patients may develop pulmonary embolism during the acute phase of deep vein thrombosis, resulting in death [20]. Post-thrombotic syndrome occurs in 23 - 60% of patients in the late stage, causing repeated or progressive limb swelling, blood stasis dermatitis, refractory skin ulcers, or limb necrosis, which seriously affect the survival and quality of life of patients [21]. Color Doppler ultrasonography is a reliable and effective method for DVT diagnosis, which has the advantages of being non-invasive and repeatable. However, imaging studies have not been able to determine the presence of fibrinolytic reactions in humans. Therefore, it has become a new research hotspot to study thrombosis from the molecular level and monitor related molecules in DVT [22].

Inflammation is key in VTE. For example, inflammation can cause damage to vein walls and induce thrombosis, thus further stimulating the obvious inflammatory response of vein walls [23]. Also, cytokines affect inflammation to a certain extent, and a high level of inflammatory factors in the blood is a risk factor for VTE [24]. Moreover, inflammatory cytokines such as IL-6/-8, and TNF-α are involved in the process of VTE [25]. IL-6 can significantly improve CD11b/CD18 expression and directly reduce CD62L transcription and translation [26]. IL-8 is directly involved in thrombus formation, accelerates the thrombolytic process, and ultimately induces neovascularization [27]. TNF-α, an inflammatory cytokine secreted by mononuclear macrophages and eosinophils, can affect the adhesion and migration of leukocytes, as well as the generation of thrombus [28]. Serum TNF-α content can reflect the degree of inflammation and tissue damage, regulate cell growth and differentiation, and control cell life activities through cell autocrine [29].

Studies on thrombus have revealed that Inflammatory factors mainly activate activation transcription factor 2 and C-transcriptase through ceramide signaling pathway, activate and bind to activator protein-1 on MMP gene thus elevating the transcription levels of the MMP genes [30]. Increased expression of MMP-2 in blood stasis and venous hypertension degrades extracellular matrix and damages the venous wall [31]. Increased MMP-16 expression has been recorded in traumatic DVT model rats [32].

In order to investigate the roles of MMP-2, MMP-16, and inflammation-associated cytokines in DVT, this research demonstrated that serum MMP-2/-16, IL-6/-8, and TNF-α in DVT patients were elevated and could be decreased after treatment. At the same time, MMP-2/-16 protein, IL-6/-8, and TNF-α mRNA in PBMCs decreased after treatment. Serum MMP-2/-16 in DVT patients were positively correlated with IL-6/-8, and TNF-α. Therefore, determining the concentrations of MMP-2, MMP-16, IL-6, IL-8, and TNF-α in the peripheral blood is of significant value in clinical practice for the diagnosis, progression, and treatment in the judgement of DVT. Future development should be concentrated on making these diagnostic markers available in a clinical setting for supportive and conclusive evidence for diagnosis of thromboembolism processes.

CONCLUSION

MMP-2/-16 and inflammatory factors are crucial in DVT, and serum MMP-2/-16 levels are positively correlated with IL-6/-8 and TNF-α levels. Therefore, the detection of MMP-2/-16, IL-6/-8, and TNF-α levels has clinical application values for the diagnosis and evaluation of the progress of DVT. However, the interaction of MMP-2/-16, IL-6/-8, and TNF-α levels in DVT remains to be further studied.

Declaration of Interest:

The authors have no conflicts of interest to declare.

Availability of Data and Materials:

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate:
The present study was approved by the Ethics Committee of Strategic Support Force Medical Center of PLA and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki, and its later amendments or comparable ethical standards.

References:

1. Saez-Gimenez B, Berastegui C, Loor K, et al. Deep vein thrombosis and pulmonary embolism after solid organ transplantation: an unresolved problem. Transplant Rev (Orlando) 2015;29(2): 85-92. (PMID: 25573688)

2. Piazza G, Goldhaber SZ. Acute pulmonary embolism: part I: epidemiology and diagnosis. Circulation 2006;114(2):e28-32. (PMID: 16831989)

3. Borgel D, Bianchini E, Lasne D, Pascreau T, Saller F. Inflammation in deep vein thrombosis: a therapeutic target? Hematology 2019;24(1):742-50. (PMID: 31736432)

4. Liu D, Zhu Y, Chen W, et al. Relationship between the inflammation/immune indexes and deep venous thrombosis (DVT) incidence rate following tibial plateau fractures. J Orthop Surg Res 2020;15(1):241. (PMID: 32616051)

5. Ma J, Cui L, Huo W, Wang G, Quan X, Zhang J. Correlation between Deep Venous Thrombosis and Inflammation in Patients after Implantation of Permanent Pacemaker. Iran J Public Health 2020;49(1):30-6. (PMID: 32309221)

6. Du T, Tan Z. Relationship between deep venous thrombosis and inflammatory cytokines in postoperative patients with malignant abdominal tumors. Braz J Med Biol Res 2014;47(11):1003-7. (PMID: 25296364)

7. Rodrigues CA, Ferrarotto R, Kalil Filho R, Novis YA, Hoff PM. Venous thromboembolism and cancer: a systematic review. J Thromb Thrombolysis 2010;30(1):67-78. (PMID: 20140479)

8. Tichelaar YI, Kluin-Nelemans HJ, Meijer K. Infections and inflammatory diseases as risk factors for venous thrombosis. A systematic review. Thromb Haemost 2012;107(5):827-37. (PMID: 22437808)

9. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90(3):251-62. (PMID: 11861412)

10. Liu YE, Wang M, Greene J, et al. Preparation and characterization of recombinant tissue inhibitor of metalloproteinase 4 (TIMP-4). J Biol Chem 1997;272(33):20479-83. (PMID: 9252358)

11. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92(8):827-39. (PMID: 12730128)

12. Yadav L, Puri N, Rastogi V, Satpute P, Ahmad R, Kaur G. Matrix metalloproteinases and cancer - roles in threat and therapy. Asian Pac J Cancer Prev 2014;15(3):1085-91. (PMID: 24606423)

13. Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol 2008;75(2):346-59. (PMID: 17678629)

14. Becattini C, Agnelli G. Pathogenesis of venous thromboembolism. Curr Opin Pulm Med 2002;8(5):360-4. (PMID: 12172436)

15. Yao Y, Shen H, Zhou Y, Yang Z, Hu T. MicroRNA-215 suppresses the proliferation, migration and invasion of non-small cell lung carcinoma cells through the downregulation of matrix metalloproteinase-16 expression. Exp Ther Med 2018;15(4):3239-46. (PMID: 29545841)

16. Zhang WL, Chen YF, Meng HZ, et al. Role of miR-155 in the regulation of MMP-16 expression in intervertebral disc degeneration. J Orthop Res 2017;35(6):1323-34. (PMID: 27227700)

17. Nakada M, Nakamura H, Ikeda E, et al. Expression and tissue localization of membrane-type 1, 2, and 3 matrix metalloproteinases in human astrocytic tumors. Am J Pathol 1999;154(2):417-28. (PMID: 10027400)

18. Strijkers RH, Cate-Hoek AJ, Bukkems SF, Wittens CH. Management of deep vein thrombosis and prevention of post-thrombotic syndrome. BMJ 2011;343:d5916. (PMID: 22042752)

19. Feng Y, Lei B, Zhang F, Niu L, Zhang H, Zhang M. Anti-inflammatory effects of simvastatin during the resolution phase of experimentally formed venous thrombi. J Investig Med 2017;65(6): 999-1007. (PMID: 28442532)

20. Giordano NJ, Jansson PS, Young MN, Hagan KA, Kabrhel C. Epidemiology, Pathophysiology, Stratification, and Natural History of Pulmonary Embolism. Tech Vasc Interv Radiol 2017; 20(3):135-40. (PMID: 29029707)

21. Appelen D, van Loo E, Prins MH, Neumann MH, Kolbach DN. Compression therapy for prevention of post-thrombotic syndrome. Cochrane Database Syst Rev 2017;9(9):Cd004174.pub3. (PMID: 28950030)

22. Komatsu H, Shimada M, Osaku D, et al. Deep vein thrombosis and serum D-dimer after pelvic lymphadenectomy in gynecological cancer. Int J Gynecol Cancer 2020;30(6):860-4. (PMID: 32276932)

23. Sullivan VV, Hawley AE, Farris DM, et al. Decrease in fibrin content of venous thrombi in selectin-deficient mice. J Surg Res 2003;109(1):1-7. (PMID: 12591228)

24. Tsai AW, Cushman M, Rosamond WD, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). Am J Med 2002;113(8):636-42. (PMID: 12505113)

25. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. J Am Coll Cardiol 2004;44(1):44-9. (PMID: 15234404)

26. Suwa T, Hogg JC, Quinlan KB, Van Eeden SF. The effect of interleukin-6 on L-selectin levels on polymorphonuclear leukocytes. Am J Physiol Heart Circ Physiol 2002;283(3):H879-84. (PMID: 12181114)

27. Henke PK, Wakefield TW, Kadell AM, et al. Interleukin-8 administration enhances venous thrombosis resolution in a rat model. J Surg Res 2001;99(1):84-91. PubMed (PMID: 11421608)

28. Eppihimer MJ, Schaub RG. P-Selectin-dependent inhibition of thrombosis during venous stasis. Arterioscler Thromb Vasc Biol 2000;20(11):2483-8. (PMID: 11073856)

29. Wisithphrom K, Windsor LJ. The effects of tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6, and transforming growth factor-beta1 on pulp fibroblast mediated collagen degradation. J Endod 2006;32(9):853-61. (PMID: 16934628)

30. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. Trends Genet 1990;6(4):121-5. (PMID: 2132731)

31. Deatrick KB, Elfline M, Baker N, et al. Postthrombotic vein wall remodeling: preliminary observations. J Vasc Surg 2011;53(1): 139-46. (PMID: 20869834)

32. Zhang YB, Li W, Yao LQ, et al. Expression changes and roles of matrix metalloproteinases in a rat model of traumatic deep vein thrombosis. Chin J Traumatol 2010;13(3):188-92. (PMID: 20515599)