

## The effect of deep venous thrombosis on oxidative damage and vitamin A and beta-carotene levels

Fevzi Sarper Türker<sup>1</sup>, Ayşe Malbora<sup>2</sup>, Mine Erişir<sup>3</sup>

<sup>1</sup>Department of Cardiovascular Surgery, University of Health Sciences, Elazığ Training and Research Hospital, Elazığ, Turkey

<sup>2</sup>Department of Physiotherapy and Rehabilitation, Bitlis Eren University, Health High School, Bitlis, Turkey

<sup>3</sup>Department of Biochemistry, Firat University Faculty of Veterinary Medicine, Elazığ, Turkey

### ABSTRACT

**Objectives:** This study aims to investigate the effect of deep venous thrombosis (DVT) on oxidative damage, vitamin A, and beta-carotene levels.

**Patients and methods:** A total of 50 DVT patients (29 females, 21 males; mean age 48 years; range, 20 to 75 years) who were admitted to the cardiovascular surgery clinic of Elazığ Training and Research Hospital with acute (<2 weeks) or chronic (≥4 weeks) DVT and 100 healthy individuals (54 females, 46 males; mean age 46 years; range, 16 to 69 years) between January 2018 and July 2018 were included. Malondialdehyde, vitamin A, and beta-carotene levels in plasma of peripheral blood samples were analyzed.

**Results:** Demographic characteristics of the acute and chronic DVT patients were similar ( $p>0.05$ ). In all DVT patients having thrombosis in the distal and proximal leg veins, the mean malondialdehyde (MDA) levels significantly increased and the mean levels of vitamin A significantly decreased, compared to healthy controls ( $p<0.05$ ). No significant difference was found in vitamin A levels and demographic characteristics between acute and chronic DVT patients ( $p>0.05$ ).

**Conclusion:** Our study results suggest that decreased vitamin A levels may play a role in the development of DVT.

**Keywords:** Beta-carotene, deep venous thrombosis, malondialdehyde, oxidative stress, vitamin A.

Venous thrombosis is one of the major causes of morbidity and mortality worldwide and occurs in one of 1,000 individuals in industrialized countries.<sup>[1]</sup> The anticoagulant therapy used for this condition is associated with bleeding side effects, although it is both preventive and therapeutic.<sup>[2]</sup> Therefore, a preventive treatment strategy is required in the follow-up of venous thrombosis without the use of anticoagulants. In the basic and research-based studies conducted for this purpose, several hypotheses have been established that vitamins can prevent venous thrombosis.<sup>[3,4]</sup> However, most of these studies have focused on arterial thrombosis.<sup>[5-7]</sup> The number of studies on venous thrombosis is very limited and, in some trials, it has been shown that vitamin D and E supplementations

can reduce the risk of venous thrombosis.<sup>[8,9]</sup> On the other hand, other studies have reported no effect.<sup>[10]</sup>

Vitamin A is a fat-soluble non-enzymatic agent and is effective in low oxygen concentrations. It has also an important role in maintaining the normal permeability and integrity of the vitamin A cell membrane.<sup>[11]</sup> All-trans retinoic acid (ATRA), the active metabolite of vitamin A, can modulate tumor-associated pro-thrombotic mechanisms considered relevant for the pathogenesis the hypercoagulable state associated with malignant disease. The vascular endothelium also plays a key role in determining the plasma fibrinolytic activity by synthesizing both tissue-type plasminogen activator (t-PA) and its specific inhibitor

Received: November 13, 2019 Accepted: February 11, 2020 Published online: March 11, 2020

**Correspondence:** Fevzi Sarper Türker, MD. SBÜ Elazığ Eğitim ve Araştırma Hastanesi Kalp ve Damar Cerrahisi Kliniği, 23280 Elazığ, Türkiye.  
e-mail: sarperTURKER@gmail.com

### Citation:

Türker FS, Malbora A, Erişir M. The effect of deep venous thrombosis on oxidative damage and vitamin A and beta-carotene levels. Turk J Vasc Surg 2020;29(3):189-195

plasminogen activator inhibitor-1 (PAI-1). Circulating t-PA, which is predominantly responsible for plasma fibrinolytic potential, is mainly derived from vascular wall, where it is localized in endothelial cells. It has been demonstrated that ATRA induces expression of t-PA in human umbilical vein endothelial cells without having a marked influence on the PAI-1 synthesis. The human t-PA gene is thought to be modulated by the retinoic acid.<sup>[12]</sup> Retinoic acid increases t-PA production by more than 2.5 times.<sup>[13]</sup>

In the literature, a limited number of vitamin A applications is available in venous thrombosis.<sup>[14]</sup> However, there is no study on the effect of vitamin A and beta ( $\beta$ )-carotene levels in the formation of venous thrombosis. According to the studies of vitamin A application, vitamin A has shown antioxidant effects in low oxygen presence,<sup>[11]</sup> and retinoic acid increases the fibrinolysis effect by increasing t-PA production.<sup>[12]</sup> In recent studies, it has been additionally reported that increased reactive oxygen species (ROS) have an important role in hypertension, arteriosclerosis, abdominal aortic aneurysms, and venous pathologies.<sup>[15]</sup> Serum malondialdehyde (MDA) is widely accepted as an indicator for lipid peroxidation and free radical activity.<sup>[16]</sup> Due to the limited researches and previous study results in this topic, we aimed to investigate the effect of deep venous thrombosis (DVT) on oxidative damage, vitamin A, and  $\beta$ -carotene levels.

## PATIENTS AND METHODS

A total of 50 DVT patients (29 females, 21 males; mean age 48 years; range, 20 to 75 years) who were admitted to the cardiovascular surgery clinic of Elazığ Training and Research Hospital with acute (<2 weeks) or chronic ( $\geq 4$  weeks) DVT and 100 healthy individuals (54 females, 46 males; mean age 46 years; range, 16 to 69 years) between January 2018 and July 2018 were included in this study. Those with chronic renal insufficiency and advanced age ( $\geq 75$  years), those receiving active cancer treatment, and long-bedridden patients were excluded. Acute DVT diagnosis was made in the outpatient or emergency setting based on the medical history, presence of positive Homans' sign and lower extremity venous Doppler ultrasound (DUS). Their DVT treatment during taking blood samples was recorded. Venous DUS results were obtained from the digital recording systems. The control group was selected by the treating physicians, nurses, and other health care personnel working in the hospital.

A written informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee of Firat University, Faculty of Medicine (No. 2017-25). The study was conducted in accordance with the principles of the Declaration of Helsinki.

## Blood sampling and biochemical analyses

Blood samples were taken from the peripheral veins into tubes containing heparin. The heparinized blood was centrifuged at 3,000 rpm for 5 min and its plasma was separated. Then, it was kept at the freezer at  $-80^{\circ}\text{C}$  until biochemical analysis.

Using the lipid peroxidation assay method, the MDA in plasma was analyzed based on the method of Placer et al.<sup>[17]</sup> with slight modifications. The MDA formed a pink complex with thiobarbituric acid (TBA), and the absorbance was read at 532 nm. The plasma MDA content was expressed in nmol/mL.

For the vitamin A and  $\beta$ -carotene analyses, vitamin A (retinol) and  $\beta$ -carotene concentrations were measured chemically. A total of 1 mL of each serum sample was mixed with 1.0 mL of ethanol containing butylated hydroxytoluene in a test tube, then 3 mL hexane was added. Tubes were shaken for 10 min and centrifuged at  $800\times g$  for 10 min. The supernatants were measured at double wavelength at 453 nm (for  $\beta$ -carotene) and at 325 nm (Vitamin A) and final concentrations were calculated by equations according to the method described by Suzuki and Katoh.<sup>[18]</sup>

## Statistical analysis

The minimum sample size yielding a power value of 80% (under 0.25 minimum difference and 0.42 standard deviation) was accepted as the

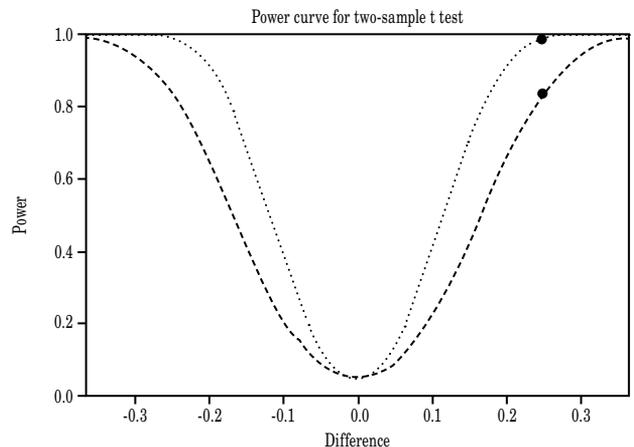


Figure 1. Power curve of the patient and control groups.

**Table 1. Demographic and clinical characteristics of deep venous thrombosis patients**

Variable	Hb		WBC		PLT×1000		ESR		CRP		AST		ALT	
	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD
Gender														
Male	26	14.7±2.6	26	6858.50±2187.61	24	247±66	24	25.62±13.72	24	1.59±1.66	26	26.69±23.06	26	25.15±22.87
Female	20	13.3±1.7	30	7310.95±4462.89	19	235±69	17	36.52±17.97	18	2.07±2.52	20	23.05±18.92	10	19.65±13.36
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05
Diabetes mellitus														
None	45	14.0±2.3	45	7078.93±3368.38	43	242±76	41	30.14±16.37	42	1.80±2.06	45	24.73±21.29	45	22.62±19.52
Yes	1	17.40	1	5990.00	0	0	0	0	0	0	1	42.00	1	29.00
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05
Hypertension														
None	40	14.1±2.4	40	7240.07±3086.39	37	236±77	36	30.69±17.04	37	1.83±2.17	40	25.77±22.54	40	23.55±20.47
Yes	6	13.8±2.1	6	5823.16±4866.29	6	279±63	5	26.20±10.77	5	1.51±0.93	6	20.66±7.63	6	17.50±7.47
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05
KOAH														
None	45	14.0±2.3	45	7078.93±3368.38	43	242±76	41	30.14±16.37	42	1.80±2.06	45	24.73±21.29	45	22.62±19.52
Yes	1	17.40	1	5990.00	0	0	0	0	0	0	1	42.00	1	29.00
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05
Smoking														
None	33	13.7±2.4	33	7257.84±3446.00	30	242±82	28	33.46±16.38	29	2.11±2.38	33	26.03±24.81	33	23.54±22.45
Yes	13	15.1±1.9	13	6341.00±3103.70	13	241±62	13	23.00±14.42	13	1.10±0.72	13	22.76±6.05	13	20.76±7.13
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05
Age (year, mean)														
<20	1	12.60	1	2300.00	1	267	1	29.00	1	1.28	1	19.00	1	9.00
<40	12	14.4±2.2	12	7029.16±1331.87	11	256±85	11	27.72±13.86	11	2.20±3.00	12	22.16±7.83	12	20.66±7.93
<60	20	14.0±2.7	20	6866.10±3406.03	20	226±58	19	26.89±14.40	19	1.76±1.80	20	21.75±6.37	20	20.50±6.51
<80	10	14.3±1.9	10	7351.00±4526.21	10	254±104	9	40.66±21.72	10	1.58±1.38	10	37.80±43.06	10	32.20±39.46
<100	1	13.60	1	6300.00	1	256	1	25.00	1	0.75	1	16.00	1	22.00
<i>p</i>		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05

SD: Standard deviation. Hb: Hemoglobin; WBC: White blood cell; PLT: Platelet; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; KOAH: Chronic obstructive pulmonary disease.

optimum sample size. The Minitab® 19 powerful statistical software (Microsoft Inc., PA, USA) was carried out for the calculation of the optimal sample size under considered conditions. Power curve is shown in Figure 1.

Statistical analysis was performed using the SPSS for Windows version 15.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in mean  $\pm$  standard deviation (SD), median (min-max), or number. For the comparison of DVT patients with healthy individuals, independent sample t-test was used. The Mann-Whitney U test was used to compare distal and proximal DVT patients versus healthy individuals. Analysis of means (ANOM) technique was used to optimize the parameters.<sup>[19,20]</sup> Demographic data of DVT patients were evaluated using the independent sample t-test. A *p* value of <0.05 was considered statistically significant.

## RESULTS

In the control group consisting of healthy individuals; three were under the age of 20, 25 people were between 21 and 30 years, 38 were between

**Table 2. Distribution of deep venous thrombosis patients according to gender and anticoagulants**

	Female (n)	Male (n)
Chronic distal DVT	2	7
Chronic proximal DVT	12	12
Acute distal DVT	2	2
Acute proximal DVT	2	11
History of pulmonary embolism		4
Anticoagulant treatment		
Low molecular weight heparin + warfarin		2
Warfarin	11	7
Non-warfarin oral anticoagulant	13	15
Low molecular weight heparin	2	4
ASA (acetylsalicylic acid)	4	4

DVT: Deep venous thrombosis; Two patients used ASA with warfarin.

**Table 3. Changes in oxidative damage, vitamin A and beta-carotene parameters between deep venous thrombosis patients and healthy controls**

Parameters	Patient	Control	<i>p</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
Malondialdehyde (nmol/mL)	2.4 $\pm$ 0.5*	1.2 $\pm$ 0.4†	<0.05 =0.000
Vitamin A ( $\mu$ g/dL)	176.1 $\pm$ 66.4*	322.3 $\pm$ 140.2†	<0.05 =0.000
Beta-caroten ( $\mu$ g/dL)	183.1 $\pm$ 41.0	174.4 $\pm$ 32.9	>0.05 =0.297

SD: Standard deviation; \*, †: The different letters in the same row show the statistical significance.

31 and 40 years, 25 were between 41 and 50 years, seven were between 51 and 60 years, and two were between 61 and 70 years. Six individuals in the control group had diabetes mellitus, four had hypertension, and 35 were smokers. Demographic characteristics were similar between the acute and chronic DVT patients. Demographic and clinical characteristics of the patients are shown in Table 1. Distribution of DVT patients according to gender and DVT therapy is summarized in Table 2.

For the comparison of all DVT patients and distal and proximal DVT patients with healthy individuals, the mean MDA levels significantly increased and the level of vitamin A significantly decreased in the

**Table 4. Changes in oxidative damage, vitamin A and beta-carotene parameters between healthy controls and patients with distal deep venous thrombosis disease (Patient 1)**

Parameters	Patient 1	Control	<i>p</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
Malondialdehyde (nmol/mL)	2.9 $\pm$ 0.3*	1.1 $\pm$ 0.5†	<0.05 =0.000
Vitamin A ( $\mu$ g/dL)	138.4 $\pm$ 28.5*	278.9 $\pm$ 54.5†	<0.05 =0.001
Beta-caroten ( $\mu$ g/dL)	163.2 $\pm$ 50.4	159.1 $\pm$ 44.5	>0.05 =0.270

SD: Standard deviation; \*, †: The different letters in the same row show the statistical significance.

**Table 5. Changes in oxidative damage, vitamin A and beta-carotene parameters between patients with deep venous thrombosis disease in proximal (Patient 2) and healthy controls**

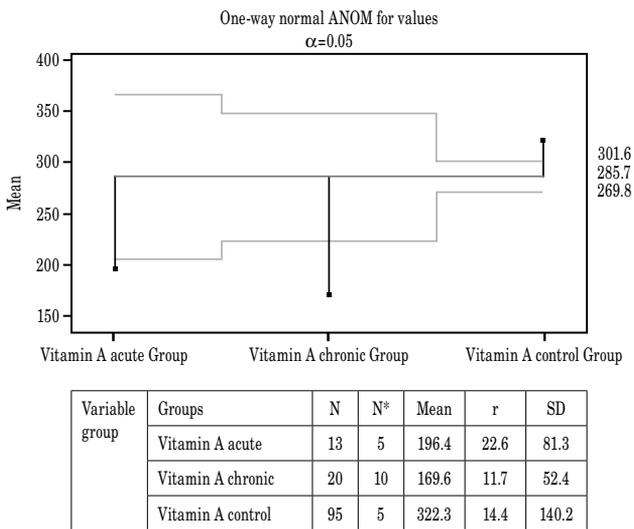
Parameters	Patient 2	Control	<i>p</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
Malondialdehyde (nmol/mL)	5.7 $\pm$ 0.3*	2.1 $\pm$ 0.4†	<0.05 =0.000
Vitamin A ( $\mu$ g/dL)	118.7 $\pm$ 32.5*	248.5 $\pm$ 60.5†	<0.05 =0.000
Beta-caroten ( $\mu$ g/dL)	164.1 $\pm$ 49.0	160.0 $\pm$ 44.0	>0.05 =0.439

SD: Standard deviation; \*, †: The different letters in the same row show the statistical significance.

**Table 6. Changes in oxidative damage, vitamin A and beta-carotene parameters between patients with distal deep venous thrombosis disease (Patient 1) with proximal deep venous thrombosis disease (Patient 2)**

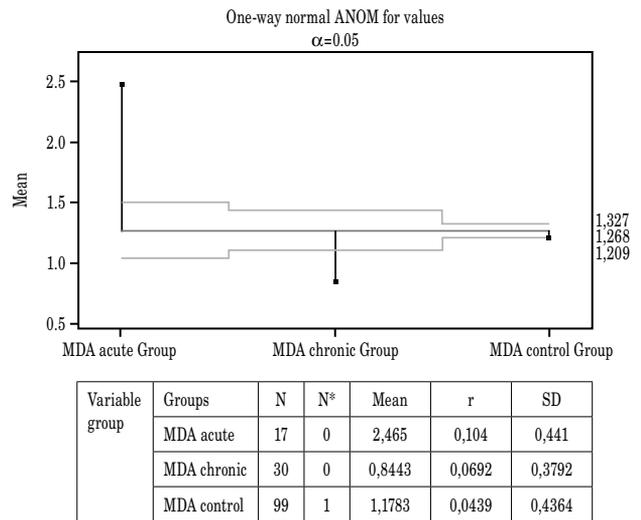
Parameters	Patient 1	Patient 2	<i>p</i>
	Mean $\pm$ SD	Mean $\pm$ SD	
Malondialdehyde (nmol/mL)	1.1 $\pm$ 0.5	1.1 $\pm$ 0.5	>0.05 =0.631
Vitamin A ( $\mu$ g/dL)	172.2 $\pm$ 37.5	174.9 $\pm$ 27.5	>0.05 =0.534
Beta-caroten ( $\mu$ g/dL)	186.0 $\pm$ 28.0	185.3 $\pm$ 37.0	>0.05 =0.872

SD: Standard deviation.



**Figure 2.** Comparison of vitamin A levels in acute and chronic deep venous thrombosis patients and healthy controls. The mean vitamin A levels of the control group were significantly higher than the acute and chronic group ( $p=0.00$ ). On the other hand, the acute and chronic groups exhibited a similar structure.

ANOM: Analysis of means; SD: Standard deviation.



**Figure 3.** Comparison of MDA levels in acute and chronic deep venous thrombosis patients and healthy controls. The mean MDA levels were significantly higher in the acute groups than the other two groups ( $p=0.00$ ). The acute group had the highest MDA levels, while the control group had the lowest levels.

ANOM: Analysis of means; SD: Standard deviation; MDA: Malondialdehyde.

patient group ( $p<0.05$ ) (Tables 3-5). No statistically significant difference in the MDA, vitamin A, and  $\beta$ -carotene levels was found between the patients with distal DVT and proximal DVT disease ( $p>0.05$ ) (Table 6). However, the mean vitamin A levels of the control group were significantly higher than the acute and chronic group ( $p=0.001$ ). On the other hand, the acute and chronic groups exhibited a similar structure (Figure 2). The levels of MDA in the acute group were significantly higher than the other two groups ( $p=0.001$ ). The MDA acute group had the highest levels, while the control group had the lowest levels (Figure 3).

## DISCUSSION

In our study, thrombosis of the deep leg veins could be related to the decreased t-PA synthesis as a result of vitamin A which was also significantly lower than healthy individuals. In addition, in all DVT patients, the patients with distal and proximal DVT disease had a significant increase in the MDA level compared to healthy individuals and this increase was considered effective in DVT disease.

Reduced plasma fibrinolytic activity is associated with increased thromboembolic events.<sup>[21,22]</sup> Human endothelial cells are an important source of t-PA and PAI-1.<sup>[23]</sup> In a study, it was shown that retinoic acid increased t-PA synthesis without changing

the production of PAI-1.<sup>[12]</sup> The induction of t-PA synthesis with retinoic acid depends on time and concentration, and micromolar concentration has been reported to cause physiological responses in animals and inhibit the colony formation of malignant cells.<sup>[24]</sup> When inducing t-PA synthesis for the protein synthesis, retinoic acid increases the synthesis in messenger ribonucleic acid.<sup>[12]</sup> Retinoids, including retinoic acid and vitamin A, are known to be effective in many biological phenomena, such as cell growth and differentiation.<sup>[12]</sup> The physiological role and potential pathophysiological condition of the t-PA induced by the synthesis of retinoic acid is important to maintain the homeostatic balance between thrombosis and thrombolysis.<sup>[12]</sup>

In many studies, it has been demonstrated that the decrease in vitamin A levels in thrombosis is effective in increasing the MDA levels. However, although previous studies mostly focused on general thrombosis, the main strength of our study is that venous thrombosis cases were included. Of note, there is a very limited number of studies on venous thrombosis in the literature. In consistent with the results of previous studies examining vitamin A and MDA, our study led us to explain the relationship between MDA and vitamin A in this patient population.

Beta-carotene is a naturally-occurring vitamin A precursor obtained from certain fruits and vegetables

with potential antineoplastic and chemopreventive activities.<sup>[25]</sup> In our study, there was no statistically significant difference in the  $\beta$ -carotene levels between the patient and control groups. However, the main limitation to this study is that there was no information about whether there was an enzymatic deficiency in the pathway converting the  $\beta$ -carotene to vitamin A. Relatively small sample size is the other limitation. In future studies, the importance of determining and using the therapeutic dose of vitamin A, which is an antioxidant in DVT patients and effective in t-PA synthesis metabolism, should be revealed in large-scale, prospective, clinical researches to develop a more effective treatment method for these patients.

In conclusion, all DVT patients including distal and proximal DVT had a significant increase in the MDA levels and a significant decrease in vitamin A levels, compared to healthy individuals. These findings highlight the importance of vitamin A in reducing the risk factors for thrombosis. Based on these study results, we speculate that decreased vitamin A levels may play a role in DVT thrombosis. Also, significantly lower levels of vitamin A in DVT patients with increased MDA levels may have contributed to the formation and progression of DVT disease with the oxidative damage it created.

#### Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

#### Funding

The authors received no financial support for the research and/or authorship of this article.

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