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Evaluation of oxidative stress balance in acute deep vein thrombosis

[Akut derin ven trombozunda oksidatif stres dengesinin değerlendirilmesi]

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ABSTRACT

Objective: The purpose of this study was to show the association between oxidative stress and deep venous thrombosis (DVT) by determining total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) values in patients with DVT and to take precautionary measures to balance oxidative status.

Methods: TOS, TAS and OSI levels in serum from 52 individuals with DVT and 45 without DVT were compared.

Results: TOS and OSI levels were significantly higher in patients with DVT compared with the control group ($p<0.0001$), but no difference was observed in TAS levels ($p=0.118$). The patient group was divided into two subgroups, idiopathic and secondary DVT. TOS and OSI levels were significantly higher patients with both idiopathic DVT and secondary DVT compared to the control group ($p<0.0001$). TAS levels were significantly higher in patients with idiopathic DVT than in the control group, but no difference was observed in the patients with secondary DVT.

Conclusion: Specificity and sensitivity values obtained from the receiver operating characteristic (ROC) curve show that TOS and OSI are both markers that can be used in the diagnosis of DVT. Also, determination of oxidative stress markers may guide clinicians to take necessary precautions to balance oxidative stress in the patients.

Key Words: Deep vein thrombosis, oxidative stress, total oxidant status, total antioxidant status

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı akut derin ven trombozlu (DVT) hastalarda toplam oksidan durum (TOS), toplam antioksidan durum (TAS) ve oksidatif stres indeksi (OSI) düzeylerini ölçerek oksidatif stres ve DVT arasındaki ilişkiyi ortaya koymak ve klinisyenlerin bu hastalarda oksidatif stresi dengelemek üzere koruyucu önlemler almasına yardımcı olmaktır.

Metod: Bu çalışmada 52 DVT'li ve 45 DVT olmayan bireylerden alınan serum örneklerinde TOS, TAS ve OSI seviyeleri belirlenerek karşılaştırıldı.

Bulgular: TOS ve OSI seviyeleri DVT hastalarında kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı yüksek bulunurken ($p<0.0001$) TAS seviyelerinde fark görülmedi, $p=0.118$. Hasta grubu idiopatik ve sekonder DVT olarak iki gruba ayrıldı. TOS ve OSI seviyeleri hem idiyopatik DVT hem de sekonder DVT hastalarında kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı yüksek bulundu ($p<0.0001$). İdiyopatik DVT hastalarında TAS seviyeleri kontrol grubundan anlamlı yüksek iken sekonder DVT hastalarında fark gözlenmedi.

Sonuç: Sonuç olarak ROC eğrisine göre elde edilen spesifite ve sensitivite değerleri TOS ve OSI parametrelerinin DVT'nin tanısında kullanılabilecek birer belirteç olabileceğini göstermektedir. Aynı zamanda oksidatif stres belirteçlerinin belirlenmesi hastalarda oksidatif stresin düzeltilmesi için klinisyenlere gerekli önlemleri alabilmeleri için yol gösterici olabilir.

Anahtar Kelimeler: Derin ven trombozu, oksidatif stres, toplam antioksidan durum, toplam oksidan durum

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

Deep venous thrombosis (DVT) is the first and the most clinical form of venous thromboembolism (VTE), a complex vascular disease associated with various etiological factors [1]. DVT affects 1-2% of society and results in serious morbidity and mortality. It imposes significant costs on the health care system and results in loss of functions and decreased quality of life in patients [2,3]. Thrombus formation and propagation depend on the presence of abnormalities of blood flow, blood vessel wall and blood clotting components, known collectively as the Virchow triad [4,5]. The processes that trigger venous thrombosis are uncertain. However, the mechanisms initiating venous thrombosis are clearly very different from those initiating arterial thrombosis. Endothelial imbalance may possibly play an important role [6]. The vascular endothelial surface normally creates a non-thrombogenic structure. When endothelial injury occurs in association with factors such as anoxia, mechanical stress, free radicals, cytokines and thrombin, this may lead to platelet activation and coagulation [7].

Oxidative stress is associated with disruption of the prooxidant and antioxidant balance [8]. It results from either overproduction of free oxygen radicals or insufficiency of antioxidant mechanisms [6]. Development of cells in an oxygen-containing environment is problematic without a strong defense system, known as the antioxidant system, which includes enzymatic and non-enzymatic components. [9]. Recent studies have shown that increased free oxygen radicals play important roles in the pathogenesis of many diseases, including hypertension, atherosclerosis and abdominal aortic aneurysm, and venous pathologies such as varicose veins, venous insufficiency and superficial thrombophlebitis [10-15]. Our scan of the literature revealed limited studies discussing the relationship between DVT and oxidative stress and antioxidant systems [6,16]. In response to increased oxidative stress, living organisms develop an antioxidant defense against the harmful effects of free oxygen radicals. Individual measurement of all the different antioxidants is difficult due to the workload involved in the laboratory, the high costs incurred, the complex techniques required and the interaction of serum antioxidants [8].

New methods have been developed for measuring all these antioxidants and oxidants expressed as a single value, such as serum total antioxidant status (TAS) and serum total oxidant status (TOS). These can be easily measured at low cost and in a very short time [17,18].

The purpose of this study was to show the association between oxidative stress and DVT by determining TOS, TAS and OSI values in patients with DVT and to take precautionary measures to balance oxidative status.

Materials and Methods

Study design and setting

Our research, planned as a prospective case-control clinical study, was carried out after receiving the necessary approval from the Ethical Committee (2012-61). Ninety-seven individuals between 20.01.2013 and 20.07.2013 attending the Department of Cardiovascular Surgery of the Ahi Evren Education and Research Hospital in Turkey between 21.01.2013 and 20.07.2013 were included.

Selection of participants

For the purpose of data collection, 52 patients presenting with lower extremity DVT symptoms including pain or swelling and diagnosed with DVT using Doppler ultrasonography were enrolled. Lower extremity acute DVT diagnosis was confirmed using Duplex Color Doppler flow imaging and compression ultrasonography (Mindray™ DC-3, 2009, Germany). Sonographic features suggestive of acute thrombosis were venous distension, a partially compressible or non-compressible lumen, a clot having a hypoechoic, homogeneous appearance, and presence of free floating thrombi. A control group of 45 volunteers selected from subjects presenting to the department with pain, swelling or minor injuries or disorders but without DVT with no exclusion criteria were enrolled as a control group.

Patients sought medical assistance with 7 days after onset of symptoms, either immediately after onset or on any other day. Patients applying after 7 days were excluded. Patients received no treatment for DVT had no previous history of it.

Our study was conducted on a voluntary basis, and volunteers meeting any of the following criteria were excluded:

1. Age less than 18
2. Cardiac disease (especially ischemic heart diseases)
3. Pulmonary embolism [19]
4. Symptoms lasting longer than seven days

Inclusion and exclusion criteria were applied to secondary DVT patients as well as to idiopathic cases. Involvement of the following deep venous segments was specified: tibial-soleal veins, the popliteal vein, the common femoral or superficial femoral vein, the iliac vein and vena cava. Specified superficial sites included the greater saphenous vein and its branches, the lesser saphenous vein and its branches, and unnamed cutaneous veins, in which case the location of the veins was specified. Each of the six deep and two superficial venous segments were graded and tabulated individually. Localization and extent of DVT was verified using duplex ultrasonography. Extension scores were grade 0=patent, 1=subsegmental, non-occlusive thrombus, 2=subsegmental, occlusive thrombus, 3=occlusive thrombus throughout length of segment. The maximal thrombotic score for a single limb was 24 [20]. DVT patients included in this study exhibited no superficial vein thrombosis.

Interventions

The 52 individuals constituting the study group were divided into two groups.

Table 1. Baseline demographic and clinical characteristics for the study and control groups

	Idiopathic DVT	Secondary DVT	Control
n	30	22	45
Female, n (%)	14 (46.7)	11 (50%)	19 (42.2%)
Age, years	54.5±16.2	61±20.3	41.8±15.1
BMI	24.3±3.1	24.6±3.7	25.4±3.3
DM, n (%)	0 (0%)	0 (0%)	1 (2.2%)
HT, n (%)	0 (0%)	0 (0%)	1 (2.2%)
COPD, n (%)	2 (6.7%)	0 (0%)	1 (2.2%)
CCF, n (%)	1 (3.3%)	2 (9.1%)	0 (0%)
CAD, n (%)	0 (0%)	1 (4.5%)	0 (0%)
PAD, n (%)	0 (0%)	0 (0%)	0 (0%)
Smoking, n (%)	3 (10%)	4 (18.2%)	6 (13.3%)
Alcohol, n (%)	0 (0%)	0 (0%)	0 (0%)
CVD, n (%)	0 (0%)	1 (4.5%)	0 (0%)
VCI Thrombosis	3 (10%)	1 (4.5%)	0 (0%)
IV Thrombosis	5 (16.7%)	3 (13.6%)	0 (0%)
FV Thrombosis	14 (46.7%)	9 (30%)	0 (0%)
PV Thrombosis	8 (26.7%)	6 (20%)	0 (0%)
TV Thrombosis	0 (0%)	3 (13.6%)	0 (0%)
Extention Score	5.4±3.1	5.4±2.3	0

BMI: Body Mass Index; DM: Diabetesmellitus; HT: Hypertension; COPD: Chronic Obstructive Pulmonary Disease; CCF: Congestive Cardiac Failure; CAD: Coronary Artery Disease; PAD: Peripheral Arterial Disease; CVD: Cerebrovascular disease.

Secondary DVT group (n=22): DVT developing after known cancer or thrombophilia, any immobilization longer than seven days, within three months of trauma or surgery, during pregnancy or new maternity or due to use of oral contraceptives is defined as secondary DVT [21].

Idiopathic DVT group (n=30): Other patients.

Baseline demographic, clinical characteristics and TAS, TOS, and OSI levels in the study population were recorded. Peripheral venous blood samples from all acute DVT patients were collected for the measurement of TAS and TOS levels before treatment within one hour of admission. For standardization purposes, all blood samples from each subject were collected in vacutainer tubes without anticoagulant by the same venipuncture staff. All blood samples were centrifuged at 1800 g for 10 min. Serum samples were stored at -80°C until biochemical assay over six months.

Measurement of total antioxidant status (TAS)

TAS levels were determined using a method developed by Erel [17] and calculated in mmol Trolox equivalent/L.

Measurement of Total Oxidant Status (TOS)

TOS levels were determined using a method previously described by Erel [18] and calculated in μmol H_2O_2 equivalent/L.

Calculation of Oxidative stress index (OSI)

The TOS/TAS ratio was used as OSI. To perform the calculation, the unit of TAS, mmolTrolox equivalent/L, was converted to μmolTrolox equivalent/L, and OSI was calculated using the formula OSI=[(TOS, μmol H_2O_2 equivalent/L) / (TAS, μmolTrolox equivalent/L) x 100].

Statistical analysis

Descriptive statistical analysis was performed for all parameters. The Kolmogorov-Smirnov test was used to determine the eligibility of variables. Data in conformity with normal distribution were analyzed using Student's t-test, and those not conforming to normal distribution using the Mann Whitney-U test. Analyses of variance test (ANOVA) or Kruskal wallis test were used for comparison of multiple groups as appropriate. Tukey's honesty for significant difference or Mann Whithney-U test were used for post hoc analyses as appropriate. Data obtained by measurements are given as mean±standard deviation. The relationship between age and biochemical parameters was determined using multivariate regression analysis. Correlations were performed with Pearson and partial correlation analysis. The area beneath the receiver operating characteristic (ROC) curves was used to determine the discriminative power of biochemical parameters in the diagnosis or exclusion of DVT. Sensitivity and specificity were calculated on the basis of ROC curves for these parameters.

Results

The research was conducted with 97 individuals between 20.01.2013 and 20.06.2013. Mean age of the 30 participants comprising the idiopathic group was 54.5±16.2 years, that of the secondary group (n=22) 61±20.3 years and that of the control group (n=45) 41.8±15.1. Baseline clinical characteristics of the study population were similar. The general clinical characteristics of the groups are shown in Table 1.

Mean time from the onset of symptoms to admission was 3.47 (0-7) days, 3.45 (0-7) days and 3.42 (1-7) days, respectively, in the idiopathic DVT, secondary DVT and control groups. Secondary causes of DVT were surgery in seven patients, immobilization in 10, malignity in four and new maternity in one. Venous thrombosis was located in the inferior vena cava in the cases, the iliac in eight, the common femoral or femoral in 23, the popliteal in 14 and the tibial vein in three (Table 1). Locations and mean extension scores in the DVT groups are shown in Table 1.

TOS, OSI and TAS levels in the control, idiopathic DVT, secondary DVT and DVT patient groups are shown in Figures 1a, b, and c. TOS and OSI levels were considerably and significantly higher in the patients with DVT compared to the individuals in the control group ($p<0.0001$). However, TAS levels did not differ significantly between the patients and controls, $p=0.118$. The patient group was classified as secondary DVT and idiopathic DVT. TOS and OSI levels were significantly higher in both the idiopathic and secondary DVT groups than in the control group (Table 2). In the idiopathic DVT group, TAS levels were higher compared with those in the control group while there was no difference between the secondary DVT and control groups (Table 2). Also there were no significant differences between the idiopathic and secondary DVT groups in terms of TOS, OSI or TAS levels (Table 2).

Results of multivariate regression analysis of TOS, TAS and age in idiopathic and secondary DVT are shown in Table 3. At multivariate regression analysis for TOS, TAS and age, TOS levels emerged as an independent predictor of idiopathic DVT and secondary DVT (odds ratio (OR): 156.18, 95% confidence interval (CI) 7.16- 3409.07, $p=0.001$; OR: 140.75, 95% CI: 6.45- 3071.01, $p=0.002$ respectively). There was no significant difference between the groups in terms of TAS and age ($p=0.837$; 0.358 for the idiopathic group) ($p=0.946$; 0.169 for the secondary group).

Optimum diagnostic cut-off point, area underneath the ROC curve (AUC), sensitivity and specificity values obtained using ROC curve analysis of TOS, TAS and OSI levels are given in Table 4.

Correlation findings between TOS, TAS and OSI levels and age and/or extension score are given in Table 5.

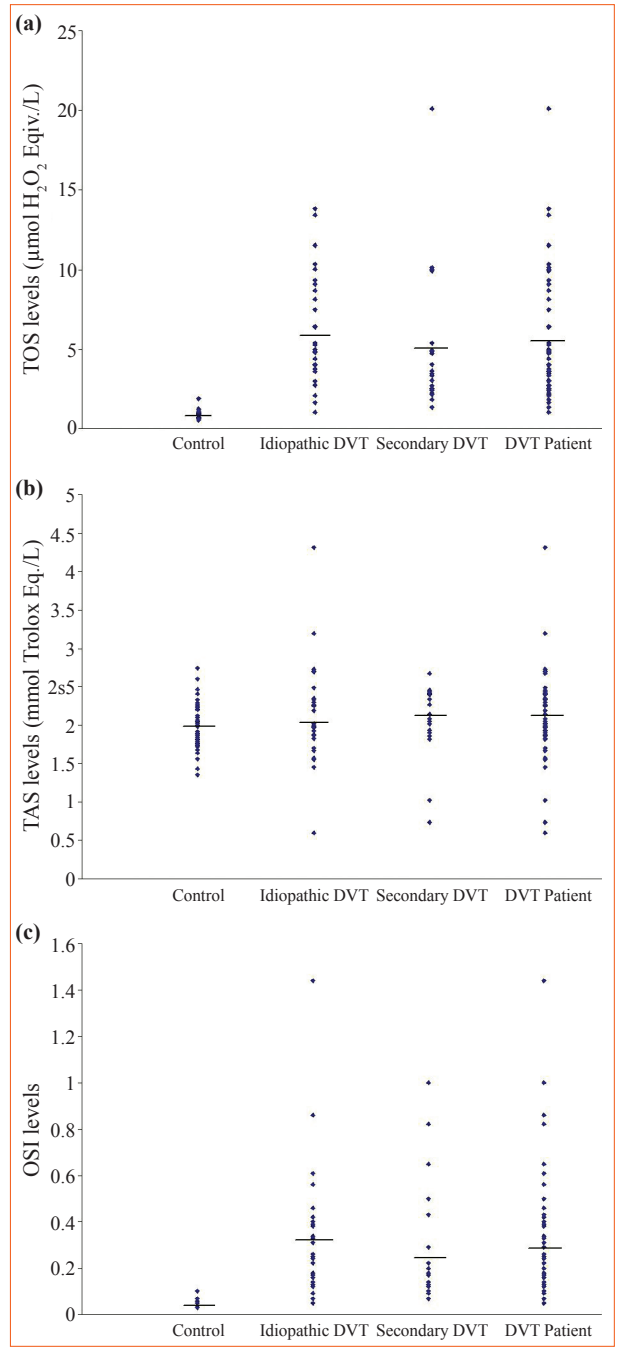

Figure 1. (a) Scattergram of TOS Levels in sera from control, idiopathic DVT, secondary DVT and DVT Patient. **(b)** Scattergram of TAS Levels in sera from control, idiopathic DVT, secondary DVT and DVT Patient. **(c)** Scattergram of OSI Levels in sera from control, idiopathic DVT, secondary DVT and DVT Patient.

Discussion

DVT is believed to be a multifactorial disease that occurs in the context of complex interactions between environmental and genetic predisposing factors. In addition, DVT is characterized by small flow velocity and a low shear rate, but a large thrombotic mass is a good model for investigating the role of oxidative stress [22]. In this study, TOS and OSI levels were considerably and significantly higher in the patients with DVT compared to the individuals in the control group ($p<0.0001$). However, there was no significant difference between TAS levels in patients

Table 2. TOS, OSI and TAS levels in the idiopathic DVT, secondary DVT and control groups

	Groups			
Parameters	Controls	Idiopathic DVT	Secondary DVT	DVT patient
n	45	30	22	52
TOS (μmol H_2O_2 Eqiv./L)	0.90±0.22	6.10±3.39[a,b]	4.94±4.3[a]	5.6±3.79[a]
OSI	0.046±0.01	0.329±0.28[a,c]	0.267±0.25[a]	0.30±0.27[a]
TAS (mmolTrolox Eq./L)	1.98±0.29	2.16±0.63[d,e]	2.08±0.45[f]	2.12±0.56[g]

[a]p=0.0001 compared with the control group; [b]p=0.440 compared with the secondary DVT group; [c]p=0.793 compared with the secondary DVT group; [d]p=0.021 compared with the control group; [e]p=0.690 compared with the secondary DVT group; [f]p=0.690 compared with the control group; [g]p=0.118 compared with the control group.

and in the control group, p=0.118. Patients with both secondary DVT and also idiopathic DVT had higher TOS and OSI values than the controls (p=0.0001). These increased levels of TOS and OSI indicate oxidative stress in patients with DVT. Regression analysis demonstrated that increased TOS levels were an independent predictor of idiopathic (p=0.001) and secondary DVT (p=0.002). Optimum diagnostic cut-off point, area underneath the ROC curve (AUC) and sensitivity and specificity values for TOS, TAS and OSI levels are given in Table 4. The table shows high sensitivity and specificity for TOS and OSI. On the basis of the ROC curve, we took the TOS and OSI levels with the highest sensitivity as cut-off values in the diagnosis of DVT (Table 4).

Various studies in the literature have shown a relationship between DVT and oxidative stress markers. Re et al. determined higher MDA, hydroxynonenal (HNE) and myeloperoxidase (MPO) levels in patients with DVT compared to healthy controls. [16]. Ekim et al. investigated MDA levels as a marker of oxidative stress and glutathione peroxidase and catalase as antioxidant enzymes in patients with DVT. They determined significantly higher MDA levels in patients with DVT compared to the individuals in the control group. Levels of antioxidant enzymes of patients were not significantly different to those of the control group, however. [6]. Mentese et al., however, demonstrated increased ischemia modified albumin (IMA) levels in a DVT group compared with a control group [23]. IMA, a Food and Drug Administration-approved serum biomarker of cardiac ischemia, appears to be an indicator of oxidative stress and may not be specific for cardiac ischemia [24].

Table 3. Multivariate regression analysis of TOS, TAS and age in idiopathic and secondary DVT

	p value	OR	CI
Idiopathic DVT			
TOS	0.001	156.18	7.16-3409
TAS	0.837	1.76	0.08-390
Age	0.358	1.047	0.95-1.15
Secondary DVT			
TOS	0.002	140.75	6.45-3071
TAS	0.946	1.20	0.01-266
Age	0.169	1.07	0.97-1.18

OR: Odds ratio, CI: Confidence interval

There is evidence suggesting that oxidative stress is involved in the pathobiology of venous thrombosis [6,16]. We encountered no studies regarding an association between TAS, TOS and OSI levels and venous thrombosis. In this study, TAS was measured as an indicator of total antioxidant capacity in the organism. We conclude that TAS levels were not altered by secondary DVT but were higher in the idiopathic DVT group than in the control group. These results show that patients with idiopathic DVT developed a greater antioxidant defense to avoid the harmful effects of increased free radicals as a response to oxidative stress. However, this increased antioxidant defense may not prevent an increase in OSI levels.

Underlying pathologies (malignancy, surgery and immobilization) may have affected the oxidative–antioxidative balance in the secondary DVT group [25,26]. Mean age of the control group was lower than that of the DVT group. Oxidative stress may increase with age [27]. Oxidative stress levels may have been affected by age in the DVT groups. TAS levels may also increase with age, however [28]. Both TOS and TAS levels were correlated with age in the control group in this study, presumably to counterbalance the increased oxidative stress. Age was not a factor for DVT, while TOS emerged as an independent factor in regression analysis. Increased TOS levels, when not compensated by TAS levels, may be the trigger of DVT.

Some authors suggest that decreased antioxidant enzymes are associated with retinal vein and central vein thrombosis and that increased oxidative stress may predispose to venous occlusive disorders or venous thrombosis [29-31]. Our study suggests that thrombosis in deep veins of the lower extremity (DVT) may be associated with oxidative imbalance. A cohort study of the high risk population is needed to improve our understanding of the effect of oxidative imbalance in DVT.

Oxidative state components may be measured separately in the laboratory, but this is time-consuming and expen-

Table 4. The optimum diagnostic TOS, TAS and TOS levels cutoff point, sensitivity and specificity according to the receiver operator characteristic (ROC) curve

	(AUC) (95% CI)	Cutoff point (%) (95% CI)	Sensitivity (%) (95% CI)	Specificity
DVT patients when compared with the control group				
TOS	0.995 (0.953-0.996)	>1.25	98 (89-99)	97 (88-99)
TAS	0.629 (0.525-0.725)	>2.25	44 (30-58)	82 (67-92)
OSI	0.991 (0.946-0.998)	>0.06	98 (89-99)	95 (84-99)
Idiopathic DVT patients when compared with the control group				
TOS	0.993 (0.938-0.996)	>1.25	96 (82-99)	97 (88-99)
TAS	0.607 (0.488-0.718)	>2.23	46 (28-65)	80 (65-90)
OSI	0.987 (0.928-0.998)	>0.06	96 (82-99)	95 (84-99)
Secondary DVT patients when compared with the control group				
TOS	0.998 (0.942-1.000)	>1.25	100 (85-100)	97 (88-99)
TAS	0.658 (0.532-0.770)	>1.9	81 (59-94)	46 (31-62)
OSI	0.996 (0.939-1.000)	>0.06	100 (84-100)	95 (84-99)
Secondary DVT patients when compared with idiopathic DVT patients				
TOS	0.658 (0.514-0.784)	>3.65	76 (57-90)	59 (36-79)
TAS	0.477 (0.337-0.620)	>2.67	16 (5-34)	100 (84-100)
OSI	0.610 (0.465-0.742)	>0.22	56 (37-74)	72 (49-89)

AUC: Area underneath the ROC curve; CI: Confidence interval.

sive. We think that measurement of TAS, TOS and OSI is more economical and faster for the diagnosis of DVT, and thus of more use to clinicians than measurement of antioxidant enzyme levels and oxidative stress markers.

Extension score was not correlated with TOS and TAS levels, so no prognostic value was established in this case control study. Response to therapy may be evaluated by monitoring TOS and TAS. Further studies are needed to determine the diagnostic and prognostic value of oxidative balance monitoring.

We predict that concomitant determination of TAS, TOS and OSI levels will be clinically useful in the rapid diagnosis of DVT. An added advantage to this strategy is that levels of these markers may potentially be used to monitor the effects of subsequent treatment. This study may help guide further research with prospective cohort studies of high-risk populations or after treatment.

We predict that concomitant determination of TAS, TOS and OSI levels will be clinically useful in the rapid diagnosis of DVT and more importantly in the treatment and care of the patients by taking necessary precautions to balance oxidative stress. An added advantage to this strategy is that levels of these markers may potentially be used to monitor the effects of subsequent treatment. This study may help guide further research with prospective cohort studies of high-risk populations or after treatment.

Table 5. Correlations between TAS, TOS and OSI and age and/or extension score

	Variables	Age	Extension score
Idiopathic DVT group			
TAS	p	0.264	0.236
	r	0.210	0.223
TOS	p	0.897	0.545
	r	-0.025	0.115
OSI	p	0.555	0.817
	r	-0.112	-0.044
Secondary DVT group			
TAS	p	0.322	0.560
	r	-0.222	-0.131
TOS	p	0.280	0.437
	r	0.241	-0.175
OSI	p	0.161	0.663
	r	0.309	-0.99
Control group			
TAS	p	0.009	
	r	0.404	
TOS	p	0.040	
	r	0.321	
OSI	p	0.576	
	r	0.090	

Limitations

The major limitation of this study is the relatively small number of patients and controls involved. Silent PE may be undetected and may have affected the results. This was a case control study and was unable to determine, by its nature, whether the oxidative stress response was triggered by DVT or was present from the very beginning.

Further studies are now needed to determine this.

Ethical approval

The study approved by judgement with 2012-61 reference number of Local Ethical Committee.

Conflict of Interest

There are no conflicts of interest among the authors.

References

[1] Mirshahi S, Soria C, Kouchakji B, Kierzek G, Borg JY, et al. New combinational assay using soluble fibrin and d-dimer determinations: a promising strategy for identifying patients with suspected venous thromboembolism. PLoS One 2014; 9(3):e92379.

[2] Goldhaber SZ. Risk factors for venous thromboembolism. J Am Coll Cardiol 2010; 56(1):1-7.

[3] Saha P, Humphries J, Modarai B, Mattock K, Waltham M, et al. Leukocytes and the natural history of deep vein thrombosis: current concepts and future directions. Arterioscler Thromb Vasc Biol 2011; 31(3):506-12.

[4] Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999; 353(9159):1167-73.

[5] Esmon CT. Basic mechanisms and pathogenesis of venous thrombosis. Blood Rev 2009; 23(5):225-9.

[6] Ekim M, Sekeroglu MR, Balahoroglu R, Ozkol H, Ekim H. Roles of the Oxidative Stress and ADMA in the Development of Deep Venous Thrombosis. Biochem Res Int 2014; 2014:703128.

[7] Balcı D. and Hazinedaroğlu S. Derin ven trombozu; epidemiyoloji, risk faktörleri, patogenez, komplikasyonlar. Türkiye Klinikleri Cerrahi Dergisi 2003; 2:81-92.

[8] Durmus A, Mentese A, Yilmaz M, Sumer A, Akalin I, et al. The thrombotic events in polycythemia vera patients may be related to increased oxidative stress. Med Princ Pract 2014; 23(3):253-8.

[9] Durmus A, Mentese A, Yilmaz M, Sumer A, Akalin I, et al. Increased oxidative stress in patients with essential thrombocythemia. Eur Rev Med Pharmacol Sci 2013; 17(21):2860-6.

[10] Glowinski J, Glowinski S. Generation of reactive oxygen metabolites by the varicose vein wall. Eur J Vasc Endovasc Surg 2002; 23(6):550-5.

[11] McCormick ML, Gavrila D, Weintraub NL. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 2007; 27(3):461-9.

[12] Uzun S, Sarıcaoğlu F, Çeliker V. Derin Ven Trombozu. Turkiye Klinikleri J Med Sci 2007; 27:853-61.

[13] Guzik B, Chwała M, Matusik P, Ludew D, Skiba D, et al. Mechanisms of increased vascular superoxide production in human varicose veins. Pol Arch Med Wewn 2011; 121(9):279-86.

[14] Krzyściak W, Kózka M. Generation of reactive oxygen species by a sufficient, insufficient and varicose vein wall. Acta Biochim Pol 2011; 58(1):89-94.

[15] Tepel M. Oxidative stress: does it play a role in the genesis of essential hypertension and hypertension of uraemia? Nephrol Dial Transplant 2003; 18(8):1439-42.

[16] Re G, Lanzarini C, Vaona I, Pazzaglia M, Palareti G, et al. Systemically circulating oxidative species in human deep venous thrombosis. Eur J Emerg Med 1998; 5(1):9-12.

[17] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37(4):277-85.

[18] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38(12):1103-11.

[19] Bounameaux H, Perrier A, Righini M. Diagnosis of venous thromboembolism: an update. Vasc Med 2010; 15(5):399-406.

[20] Porter JM, Moneta GL. Reporting standards in venous disease: an update. International Consensus Committee on Chronic Venous Disease. J Vasc Surg 1995; 21(4):635-45.

[21] Jezovnik MK, Poredos P, Lusa L. Idiopathic venous thrombosis is associated with preclinical atherosclerosis. J Atheroscler Thromb 2010; 17(3):304-11.

[22] Kamat GV, Metgud SC, Pattanshetti VM, Godhi AS. A cross-sectional study to detect the prevalence of hyperhomocysteinemia in cases of deep vein thrombosis. Indian J Surg 2010; 72(4):323-6.

[23] Mentese A, Mentese U, Turedi S, Gunduz A, Karahan SC, et al. Effect of deep vein thrombosis on ischaemia-modified albumin levels. Emerg Med J 2008; 25(12):811-4.

[24] Turedi S, Gunduz A, Mentese A, Karahan SC, Yilmaz SE, et al. Value of ischemia-modified albumin in the diagnosis of pulmonary embolism. Am J Emerg Med 2007; 25(7):770-3.

[25] Bourgeais J, Gouilleux-Gruart V, Gouilleux F1. Oxidative metabolism in cancer: A STAT affair? JAKSTAT 2013; 2(4):e25764.

[26] Hong IS, Lee HY, Kim HP. Anti-oxidative effects of Rooibos tea (Aspalathus linearis) on immobilization-induced oxidative stress in rat brain. PLoS One 2014; 9(1):e87061.

[27] Jha R, Rizvi SI. Carbonyl formation in erythrocyte membrane proteins during aging in humans. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2011; 155(1):39-42.

[28] da Cruz AC, Petronilho F, Heluany CC, Vuolo F, Miguel SP, et al. Oxidative stress and aging: correlation with clinical parameters. Aging Clin Exp Res 2014; 26(1):7-12.

[29] Angayarkanni N, Barathi S, Seethalakshmi T, Punitham R, Sivaramakrishna R, et al. Serum PON1 arylesterase activity in relation to hyperhomocysteinaemia and oxidative stress in young adult central retinal venous occlusion patients. Eye (Lond) 2008; 22(7):969-74.

[30] Bovill EG, van der Vliet A. Venous valvular stasis-associated hypoxia and thrombosis: what is the link? Annu Rev Physiol 2011; 73:527-45.

[31] Voetsch B, Jin RC, Bierl C, Deus-Silva L, Camargo EC, et al. Role of promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene as a risk factor for cerebral venous thrombosis. Stroke 2008; 39(2):303-7.