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Evaluation of some selected clotting and fibrinolytic markers in menopausal subjects suffering from venous thrombosis

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ABSTRACT

Objective: To assess some selected parameters of coagulation and fibrinolysis systems in menopausal females suffering from deep vein thrombosis (DVT), being users or non-users of hormone replacement therapy (HRT).
Design: Prospective randomised controlled study.
Setting: Patients enrolled in the Department of Ultrasonography in Polish Mother's Memorial Hospital, Research Institute in Lodz.
Patient: The population of 162 menopausal women divided into three group : 1/HRT users with deep vein thrombosis (DVT) - group H; 2/HRT non-users with DVT - group T; 3/HRT users without DVT - group C (controls).
Interventions: 1/Ultrasound (US) examinations of the venous system 2/ Blood samples to determine some coagulation and fibrinolytic parameters.
Main Outcome Measure: 1/The finding of DVT in US study. 2/In Blood: platelet count and function, prothrombin ratio and time, INR, APTT, thrombin time, fibrinogen, factor VII, VWF, D-Dinner, TAT, ProC Global, AT III, Protein C, protein S, PAI-1, t-PA and LA.
Results: In the group H we observed: heigher INR values, increased activities of VWF, lower activities of protein C with increased D-dinner concentrations. However, the group T was characterized by: shortened platelet closure time (EPI), higher INR values, elongated APTT, higher VWF activities, lower ProC Global rations with higher activities of AT III and lower activities of protein S. Both groups presented also high levels of fibrinogen, PAI-1 and TAT complexes.
Conclusions: The observed disorders in coagulation and fibrinolysis system in menopausal women with detected DVT should be the reason to look for thrombophilia in this group of patients.
Key words: Hormone replacement therapy, thrombophilia, thrombophlebitis.

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INTRODUCTION

Women using oral oestrogen containing hormone replacement therapy or oral contraceptives have an increased risk of developing venous thrombosis.
Oral contraceptive use was first associated with thrombosis in 1961, with a report of pulmonary embolism in a nurse, who had just begun taking an oral contraceptive containing 100 μgmestranol for the treatment of endometriosis.[1] In1963, the first case of myocardial infarction in an oral contraceptive user was reported.[2] Estrogens increase the risk of venous thrombosis when used as oral contraceptive or as

postmenopausal hormone replacement.[3-5] A similar effect was observed in men when estrogens were used as a treatment of coronary disease[6] or in sex-change treatment.[7] Recently, it has been demonstrated that the progestins in combination with oral contraceptives also affect the risk of thrombosis.[8-10]

Thombosis is a serious disorder. Venous thrombosis, although rarely fatal, often leads to a disabling postthrombotic syndrome.[11] Arterial thrombosis may be fatal, as myocardial infarction, or lead to disabling sequelae in stroke.

The risk factors for thrombosis can be divided into 3 groups of causes, according to Virchow[12]: reduced blood flow, changes in the vessel wall, and changes in the composition of the blood. In venous thrombosis, causes related to stasis and blood coagulability are most important. whereas in arterial disease, vessel wall changes stand out. Genetic risk factors for venous thrombosis lead to hypercoagulability, whereas the acquired causes are either associated with decreased flow (as found in immobilization, paralysis, surgery, and plaster casts) or related to blood coagulation (associated with the lupus anticoagulant, pregnancy, oral contraception, and malignancies).

In an early study of adverse effects of estrogen replacement therapy, a slight risk increase of venous thrombosis was observed.[13] This was not confirmed in subsequent studies.[14-16] and the idea estrogen replacement could cause venous thrombosis was dismissed as "medical superstition.[17] However, from 1996 onward, a series of studies has demonstrated that hormone replacement users have a 2- to 4-fold increased risk fo venous thrombosis.[3-5, 18-23]

Risk of thromobosis is highest in the first year of use. [3,4,20,21] and in some studies.[19,20,23] but not in all.[3,22] the risk was limited to the first year. Oral use and transdermal patches increase the risk of thrombosis.[19,20] and an association with thrombosis has been found for conjugated estrogens as well as for estradiol.[19,23]

Why did the early studies not detect the thrombosis risk associated with thrombosis? This may have been caused by less reliable diagnostic methods in the past, which may have been adequate to detect the risk of the then high-dosed oral contraceptives but not of the lower estrogen dose in hormonal replacement therapy. The less widespread use of hormone replacement may also have been a factor.

Venous thromboembolism (VTE) constitutes the most severe complication of hormone replacement therapy (HET). From the published analyses it appears that the highest risk for VTE is found during the first year of HRT: a relative risk of VTE in the first twelve months of that therapy was calculated as 6.7, then being reduced during the next years to 1.9-2.8 [3,4] But even despite the latest results coming from the Women's Health Initiative (WHI) there will be still a substantial population of menopausal females requiring the HRT administration for a short or longer lifetime span.

The mechanism of VTE isn't still accurately recognized. In medical literature more attention is paid to the issue of thrombus formation within arterial vessels.[24,25]

Menopausal age itself is characterized by some negative alterations in haemostasis and, thereby, by the increased risk of venous thrombosis. Apart from the presence of many different VTE risk factors resulting in reduced blood flow in peripheral vessels, vascular wall damage and impaired endothelial function, it is also claimed to be a period of unfavourable prothrombotic and anti-fibrinolytic changes in blood clotting. [26-28]

Therefore, the aim of this present study was to assess some selected parameters of coagulation system in menopausal women suffering from venous thrombosis and being users or non-users of hormonal substitutive therapy.

Methods

The study population consisted of 162 women at menopausal age (range: 45-69 yea; mean age 51±10 yrs) qualified for the assessment of coagulation/ fibrinolysis systems by the Department of Ultrasonography located in Polish Mother's Memorial Hospital, Lodz. In 78 females, being HRT users for at least three months, no signs of deep vein thrombosis (DVT) were detected in ultrasound examination of lower limb venous system, which was confirmed in the second examination after 5-6 months (controls - group C). However in 84 women some ultrasound features of the presence of DVT were revealed. From among these DVT women 53 patients were non-users of HRT - group T. The rest of these subjects (n=31) were taking HRT at the moment of the onset of thrombosis - group H. The characteristic of the study population is pressented in Table 1.

Ultrasound examinations of the venous system were performed in women at menopausal age with a clinical suspicion of being DVT patients. Our anamnestic data base included hormonal therapy (duration, dose and regularity of HRT) and other risk factors of thrombosis. The diagnosis of venous thrombosis has been done on the basis of ultrasound examination made in a colour Doppler technique (USG -CD). While we evaluated inferior caval vein and iliac vessels, some 2-4MHz broad-band convex probes were utilized, as well as some other 5-12 MHz linear probes in the peripheral vessels' examination. The ultrasound procedure have been conducted according to the standards in the evaluation of venous system.[29,30] A diagnosis of thrombosis followed mostly the compression test. Whereas the evaluation of iliac veins and deeply located vessels of the limb was completed with the observation of blood flow in the course of the limb's distal compression and the Valsalva test.

Table 1. Characteristics of patients recruited for this study.

Group	Age (in years) (mean ± SD)	HRT	DVT
H (n = 31)	50.3 ± 10.5	Yes	Yes
T (n = 53)	49.5 ± 12.7	No	Yes
C (n = 78)	52.4 ± 5.2	Yes	No

group C = control group

Table 2. Haemostatic parameters in serum of patients from all the three groups (mean values ± SD).

Parameter (range)	Group H	Group T	Group C
PLTx 10 (150-350)	221.1 ± 78.3	225.5 ± 78.8	205.4 ± 194.5
Platelet closure time - EPI (sec.) (85-165)	159.4 ± 70.0	**148.3 ± 66***	171.2 ± 70.2
Platelet closure time - ADP (sec.) (71-118)	92.7 ± 25	96.9 ± 44.9	103.2 ± 31.1
Prothrombin index (%) (80-120)	99.69 ± 41.62	82.62 ± 26.18	96.77 ± 14.41
Prothrombin time (sec.) (11.2-16.0)	13.86 ± 6.21	13.91 ± 13.95	15.82 ± 8.51
INR (0.8-1.2)	**1.28 ± 0.81***	**1.48 ± 0.85****	1.06 ± 0.15
APTT (sec.) (23-36)	31.70 ± 6.51	**34.67 ± 9.07***	31.47 ± 6.41
Thrombin time (sec.) (16-21)	16.29 ± 2.70	17.28 ± 2.45	17.17 ± 2.32
Fibrinogen (g/l) (1.8-3.5)	3.65 ± 0.85	3.77 ± 1.19	3.40 ± 0.89
Factor VII (%) (70-125)	105.12 ± 53.55	107.6 ± 52.89	110.16 ± 51.76
VWF (%) (50-150)	**125.0 ± 28.7****	**115.7 ± 30.2***	106.7 ± 33.6
ATIII (%) (75-125)	103.3 ± 9.4	**102.7 ± 10.7***	99.9 ± 12.2
ProC Global (R) (1.7-2.0)	1.79 ± 0.39	**1.72 ± 0.51****	2.07 ± 0.45
Protein C (%) (70-140)	**116.1 ± 36.3***	121.4 ± 41.6	130.6 ± 23.0
Protein S (%) (70-123)	60.3 ± 29.9	**56.8 ± 43.6***	73.5 ± 48.0
PAI-I (ng/1) (45±33)	93.65 ± 28.53	105.27 ± 32.17	97.75 ± 34.89
t-PA (ng/1) (1-12)	4.76 ± 2.89	6.07 ± 4.46	5.18 ± 3.34
LA (fraction) (0-1.2)	1.26 ± 0.2	1.25 ± 0.4	1.25 ± 0.2
D-dimer (meg/1) (0-200)	**504.4 ± 708.2***	356.4 ± 46.3	252.0 ± 384.4
TAT (μg/1) (1.0-4.1)	7.93 ± 10.18	9.84 ± 19.84	7.72 ± 14.52

p≤0.05*, p<0.01**ß

A thorough assessment of coagulation and fibrinolysis systems was performed at the same time: in blood samples (4.4 ml of venous blood) taken from every female patient the following parameters were under our evaluation: 1/platelet count (10μl of the blood, the platelet analyser - Baker 810); 2/platelet closure time - EPI and ADP (the PFA-100 system, Dade-Behring), 3-5/ prothrombin time, prothrombin ratio and INR (clotting assay, Thromborel S kits - Dade Behring); 6/ APTT (clotting assay, Pathromtin SL kits - Dade Behring); 7/ thrombin time (clotting method, BC Thrombin Reagent - Dade Behring); 8/ fibrinogen (clotting assay, Multifibren U kits - Dade Behring); 9/ factor VII (clotting assay, Coagulation Factor VII Deficient Plasma (human) kits - Dade Behring); 10/ VWF (clotting method, BC von Willebrand Reagent - Dade Behring); 11/ D-Dimer (quantative method, Turbiquant D-dine kits - Dade Behring); 12/ thrombin-antithrombin III complexes - TAT(ELISA technique, Enzygnost TAT micro kits - Dade Behring); 13/ anticoagulatory capacity of protein C system in human plasma - ProC Global (clotting method, ProC Global kits - Dade Behring); 14/ AT III (chromogenic method, Berichrom Antithrombin III kits - Dade Behring)' 15/ protein C (chromogenic method, Berichrom Protein C kits - Dade Behring); 16/ protein S (clotting assay, Protein S Reagent kits - Dade Behring); 17/ PAI-1 (ELISA technique, coalisa PAI-1 kits - Chromogenix); 18/ t-PA (ELISA techique, Coalisa t-PA kits - Chromogenix); and 19/ lupus anticoagulant - LA (clotting assay, LA and LA 2 kits, Dade Behring).

The subsequent statistical analysis of the obtained parameters included variance analysis, the Student t-test and the Mann-Whitney non-parametric U-test. For all the parameters, arithmetical averages and standard deviations were calculated.

Results

The following statistically signiflcant differences, as compared to the controls, of the studied parameters were observed:

In women of group H who were on HRT at the onset of VT: 1/ INR was higher (1.28 ± 0.81 and 1.06 ± 0.15 respectively, $p<0.05$); 2/ activities of VWF were higher (125.0 ± 28.7 and 106.7 ± 33.6% respectively, $p<0.01$); 3/ activities of protein C were lower (116.1 ± 36.3 and 130.6 ± 23.0% respectively, $p<0.05$); 4/ D-dimer concentrations were increased (504.4 ± 708.2 and 252.0 ± 384.4 ng/dl respectively, $p<0.05$).

In group T who were women with VT without evidence of hormone replacement therapy it was observed: 1/ shortened platelet closure time (EPI) (148.3 ± 66 and 171.2 ± 70.2 sec. respectively, $p<0.05$); 2/ higher INR values (1.48 ± 0.85 and 1.06 ± 0.15respectively, $p<0.01$); 3/ elongated APTT (34.67 ± 9.07 and 31.47 ± 6.41 sec. respectively, $p<0.05$); 4/ higher VWF activities (115.7 ± 30.2 and 106.7 ± 33.6% respectively, $p<0.05$); 5/ lower ProC Global rations (1.72 ± 0.51 and 2.07 ± 0.45 respectively, $p<0.01$); 6/ higher activities of AT III (102.7 ± 10.7 and 99.9 ± 12.2% respectively, $p<0.05$); 7/ lower activities of protein S (56.8 ± 43.6 and 73.5 ± 48.0% respectively, $p<0.05$). The subjects with thrombosis exhibited high fibrinogen concentrations. Also the levels of PAI-1 and TAT were above the normal range in all these women. The results of haemostatic parameters in the 3 groups studied is presented in Table 2.

Discussion

The maintenance of haemostasis needs a balance between both coagulation/fibrinolysis systems and their regulating inhibitors. Disturbing the relation between some components involved in thrombus generation/degradation processes, e.g. an increase in the concentration of clotting factors accompanied by decreased levels of endogenous anticoagulants or a drop of both plasminogen and their activators with increased levels of fibrinolytic inhibitors, may lead to the formation of thrombi within the vascular lumina which then proceeds to the development of venous thromboembolism[31,32]

The results of our study suggest a possible causal relationship between current HRT use and idiopathic VTE. This interpretation is supported by results from a study of exogenous hormones in relation to risk of pulmonary embolism by Grodstein and colleagues.[5]

In the patients with thrombosis from our study we have observed some clear pro-thrombotic shifts in the coagulation system consisting in: 1/ the rise of von Willebrand factor activity (more distrnct in group H) and 2/impaired anticoagulative properties of serum (mostly group T - lower ProC Global values, lower activity of protein S).

Intravasular activation of coagulation and fibrinolysis manifests itself in the form of some markers of haemostasis activation appearing in blood (e.g. D-dimer, TAT, F_{1+2} FDP, PCP, PAP, Bβ 1-42). All these parameters testify to the functional status of endothelium, early platelet-depended activation of coagulation, intravascular thrombin/fibrin generation and fibrinolytic system status. [33]

The evaluation of platelet function in the groups with thrombosis revealed shorter platelet closure time in the group without HRT (platelet closure time a parameter describing in-vitro-stimulated processes accompanying vasular wall damage such as platelet activation and aggregation). However, increased D-dimer concentrations observed only in group H may testify to the earlier stage of detected thrombosis (and a more thorough medical surveillance as well) in these patients.

Studies on the influence of HRT on coagulation system aren't unquivocal. Some authors suggest pro-coagulative action of sex steroids, increasing factor

VII levels, decreasing endogenous inhibitors activities, while some others are testifying to decreased levels of factor VII and fibrinogen.[34-37]

Fibrinogen is a chief factor influencing blood viscosity - its enhanced level deteriorates blood flow conditions in vessels. From the hitherto existing data we already know that increased concentrations of fibrinogen are conducive to thrombotic lesions both in venous and arterial vessels. [38,39] Our results also corroborate the above reported findings.

However, identifying a factor responsible directly for evoking the pathological process turned out to be impossible in 50% of patients with DVT. It is probably the result of the coexistence of several factors, among others some hereditary defects of haemostatic system being still evaluated. It has been proven that DVT is also caused by the presence of other factors, e.g. hyperhomocysteinemia.[40,41]

In both groups, most of the women, apart from age and oestrogens, were saddled with subsequent risk factors of VTE. Only in some of them there was a possibility to identify thrombosis (injury, operative procedures or immobilization during one month preceding occurrence of the disease).

Our data supports an association between estrogen replacement therapy and venous thromboembolism, although many questions remain. The pathophysiology is not well understood; however, the effect of estrogen on the vascular endothelium and on coagulation factors might affect the potential for a thromboembolic event.[42,43] These hypercoagulable states might also be opposed by properties of estrogen-induced clot lysis, and an imbalance in these processes in some women might result in thromboembolism.[44] A follow-up analysis of the PEPI trial observed that patients with venous thromboembolism had lower baseline fibrinogen levels than patients without venous thromboembolism.[45] The significance of these findings is unclear. An Italian study suggests that continuous transdermal estradiol use results in better hemostatic balance of clotting factors than cyclic estradiol therapy.[46]

Identification of persons at highest risk requires further investigation. The HERS trial[47] reported increased risk in patients with hip or lower-extremity fracture, cancer, hospitalization, or surgery. Other expected risk factors (hypertension, smoking, or body mass index) did not predict venous thromboembolic events. Later onset of menopause (.52 years of age) was also associated with increased risk. Use of statin medications and use of aspirin had protective effects. However, whether all of these findings can be extrapolated to women without coronary artery disease is unclear. The Estrogen in Venous Thromboembolism Trial (EVTET) reported that women with a history of venous thromboembolism using estrogen replacement therapy are at increased risk for a recurrent event .[48] Women with the factor V Leiden mutation who use estrogen are also at increased risk for atherothrombolic events[49] and venous thromboembolic events.[50] Further study must determine when to screen for coagulopathies in postmenopausal women before starting estrogen replacement therapy.

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