

RISK FACTORS OF VENOUS THROMBOEMBOLISM IN SUDANESE PREGNANT WOMEN

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ABSTRACT

Venous thromboembolism (VTE) is one of the major causes of pregnancy-related mortality and morbidity. This study aimed to determine the frequency of factor V Leiden (FVL) and prothrombin G20210A polymorphisms and measure the plasma levels of protein C (PC), protein S (PS) and antithrombin (AT) in pregnant women with VTE and healthy pregnant women. This prospective case-control study determined the frequencies of FVL G1691A and prothrombin G20210A polymorphisms and measured the plasma levels of PC, PS and AT in 198 pregnant women with VTE and 198 healthy pregnant women. Allele-specific polymerase chain reaction (ASPCR) was used to detect the FVL G1691A polymorphisms and prothrombin G20210A gene mutations. The FVL G1691A polymorphism and prothrombin G20210A gene mutations were detected only in pregnant women with VTE, with frequencies of 4.0 and 0.5%, respectively. The highest frequency of FVL G1691A polymorphism was observed in patients with deep vein thrombosis (DVT) and positively associated with contraceptive use and termination. Pregnant women with VTE had significantly lower levels of PC, PS and AT than those of controls. In conclusion, among the VTE cases, FVL G1691A polymorphism and PC, PS and AT deficiencies were the most common findings in patients

presenting with DVT. Antithrombin deficiency was more common than PC and PS deficiencies. Contraceptive use, high body mass index (BMI) and termination correlated strongly with FVL G1691A polymorphism and PC and PS deficiencies in patients with VTE.

Keywords: Deep vein thrombosis (DVT); Factor V Leiden (FVL); Protein C (PC); Protein S (PS); Prothrombin G20210A polymorphism.

INTRODUCTION

Venous thromboembolism (VTE), manifested as deep vein thrombosis (DVT) and/or pulmonary embolism (PE), is one of the major causes of pregnancy-related mortality and morbidity [1]. The annual incidence of VTE ranges from one to five per 1000 individuals; however, its diagnosis remains challenging and difficult [2].

Venous thromboembolism may occur due to inherited disorders or be associated with acquired clinical condition(s). The prominent etiologies of VTE in pregnancy are inherited thrombophilias, which include deficiencies of protein C (PC), protein S (PS) and antithrombin (AT). Factor V Leiden (FVL G1691A) polymorphism, prothrombin G20210A, and methylenetetrahydrofolate reductase gene mutations also cause VTE in pregnancy [3,4]. Acquired clinical conditions that may result in VTE in pregnancy include autoimmune and inflammatory disorders, increased body mass index (BMI), obesity, prolonged immobilization, miscarriages, surgical intervention, contraceptive use and multiparity [4-6].

During pregnancy, which is a physiological hypercoagulable state, there is also increased activity of coagulation factors I, II, VII, VIII, IX and X and tissue factor [7]. Plasma level of PS, activated PC levels, and plasminogen activator inhibitor levels decrease in pregnancy [7]. Thrombophilia further aggravates the preexisting hypercoagulable state in pregnancy [3]. In pregnant women, the

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risk of VTE has been reported to be 5- to 6-fold compared with that in nonpregnant age-matched controls [1,8]. This risk further increases from 3.7- to 8.5-fold in women with a family history of VTE and up to 34-fold in women with hereditary thrombophilia [1]. The clinical manifestations associated with the FVL G1691A polymorphism in pregnant women include severe placental abruption, fetal growth disturbances, placental infarction, stillbirth, increased risk for gestational hypertension, hemolysis, elevated liver enzymes, and low platelet syndrome [9]. Considering the clinical manifestations of thrombophilia, this study aimed to determine the frequencies of FVL G1691A polymorphism and prothrombin G20210A mutations and measure the plasma levels of PC, PS and AT in pregnant women with VTE and healthy pregnant women.

MATERIAL AND METHODS

Study Design. This prospective case-control study was conducted between January 2018 and August 2019 at the Khartoum Teaching Hospital in Khartoum State, Sudan, recruiting 396 pregnant women. Ethics approval was obtained from the University of Science and Technology, Sudan, and the study was conducted according to the Declaration of Helsinki. Informed consent was obtained from all women before the collection of blood samples. Blood samples were collected before the initiation of the anticoagulant therapy. The 396 participants were equally divided into pregnant women diagnosed with VTE ($n = 198$) and normal pregnant women ($n = 198$) without any history or diagnosis of VTE as age-matched controls. The diagnosis of VTE was based on the presence of clinical signs suggestive of VTE and confirmed by venous compression ultra-sonography and/or multidetector-row (spiral) computed tomography.

Data were collected using a predesigned and pre-tested questionnaire. Participants were asked about demographic data and VTE risk factors, such as familial history of thrombosis, fractures, recent surgery, kidney disease, malignant tumors, immobilization, history of hypertension, diabetes mellitus, smoking habits, and use of oral contraception or hormone replacement therapy. The BMI was calculated to evaluate obesity [body weight (kg) divided by the square of height (m)].

Plasma Extraction and Analysis. Blood samples were collected in EDTA-containing vacutainers for DNA analysis and in sodium citrate-anticoagulated tubes for PC, PS and AT measurements. Citrated plasma was obtained by centrifugation of blood samples at 3000 rpm for 30 min. Plasma was extracted and stored at -80°C until analysis. Protein C and PS levels were measured by a clot-based assay using the reagents of TECHNOCLOT® (Technoclone

Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria). These levels were determined from a calibration curve in which prolongation of clotting time is proportional to the protein concentration. The AT level was determined using an automated coagulation analyzer (Sysmex CA-1500) using the TECHNOCHROM® AT III Kit (Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH). Reference values of PC, PS and AT in the range of 55.0-125.0, 50.0-130.0 and 62.0-120.0 ng/mL, respectively, were considered as normal based upon the preestablished normal levels. A decreased level of any of these natural anticoagulants from the reference values were considered to be deficient.

DNA Extraction and Analysis. DNA was extracted from the blood samples collected in the EDTA vacutainers and purified using the QIAamp DNA Blood Mini Kit according to the manufacturer's standard protocol (Qiagen Sciences Inc., Germantown, MD, USA). The FVL G1691A polymorphism and prothrombin G20210A mutation were detected by allele-specific polymerase chain reaction (ASPCR) using the Bio-Rad DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Foster City, CA, USA) as described previously [10].

Statistical Analysis. Qualitative data, which are expressed as proportions, were analyzed using the χ^2 test. Quantitative data are expressed as mean \pm SD. The z-test was used for testing the significance of quantitative data. The association between two quantitative parameters was evaluated by correlation analysis, and adjusted odds ratios (ODs) were calculated for determining the strength of association between qualitative parameters. Statistical significance was set at $p < 0.05$.

RESULTS

Among the 396 study participants, 198 were pregnant women with VTE (VTE group) and the remaining 198 were healthy pregnant women (control group), with the mean (\pm SD) age being 28.21 ± 7.69 and 28.42 ± 5.93 years ($p > 0.05$), respectively (data not shown in table). Among the VTE group, DVT was the most common clinical feature ($n = 156$, 78.8%), followed by pulmonary thromboembolism ($n = 20$, 10.1%). The frequencies of venous thrombosis of the upper limbs, portal vein, and cerebral vein were 5.1, 4.0 and 2.0%, respectively (Table 1).

A total of 161 patients (81.3%) showed an association with various known risk factors of VTE, whereas 37 patients (18.6%) had no known risk factor of VTE (Table 2). Patients with a history of contraceptive use were more susceptible to developing VTE (33.8%). Moreover, high BMI (12.6%), termination (11.6%) and family history (10.1%), were the most common risk factors of VTE in

Table 1. Clinical features of pregnant women with venous thromboembolism (*n* = 198).

Clinical Features	Frequency	%
Deep vein thrombosis	156	78.8
Pulmonary thromboembolism	20	10.1
Upper limb venous thrombosis	10	5.1
Portal vein thrombosis	8	4.0
Cerebral vein thrombosis	4	2.0

Table 2. Risk factors of venous thromboembolism in pregnant women (*n* = 198).

Risk Factor	Frequency	%
Contraceptive use	67	33.80
High body mass index	25	12.60
Termination	23	11.60
Family history	20	10.10
Combined risk factors	14	7.07
Hypertension	6	3.03
Surgery	3	1.52
Renal disease	2	1.01
Immobility	1	0.51
Smoking	0	0.00
Trauma	0	0.00
No known risk factors	37	18.69

the study population. The frequencies of other risk factors are shown in Table 2.

Thrombophilia profiles revealed highly significant differences in the VTE group compared with those in the control group (*p* < 0.05). The VTE group had significantly lower levels of PC (74.93 ± 40.8 vs. 97.83 ± 23.75 ng/mL) and PS (87.47 ± 25.24 vs. 102.46 ± 22.73 ng/mL) than those in the control group (*p* < 0.01). Although, AT levels were slightly lower in the VTE group (80.19 ± 30.33 ng/mL) than in the control group (85.46 ± 18.23 ng/mL), the differences were statistically significant (*p* < 0.05) (Table 3). The frequency of PC deficiency was 6.7% in the VTE group compared with 1.01% in the control group. The frequency of PS deficiency in patients with VTE was also higher than that in the control group (Table 3). Moreover, AT deficiency was the most common abnormality among patients with VTE (11.6%) and controls (5.1%) (Table 3).

Regarding FVL G1691A, the frequency of the homozygous normal genotype (G/G) was lower in the VTE group (96.0%) than in the control group (100.0%). Heterozygous (G/A) and homozygous (A/A) mutations were detected in the VTE group, with frequencies of 3.5% (*n* = 7) and 0.5% (*n* = 1), respectively (Table 4). The prothrombin G20210A mutation exhibited a statistically insignificant difference between the VTE and control groups (*p* = 0.499). Heterozygous (G/A) prothrombin G20210A mutation was found in only one patient (0.5%), and none

Table 3. Levels and frequencies of protein C, protein S and antithrombin in the study participants (*n* = 396).

Variable Features	VTE Cases Mean±SD	Controls Mean±SD	<i>p</i> Value	Status	VTE Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR (95% CI)	<i>p</i> Value
Protein C	74.93±40.8	97.83±23.75	0.0001	deficient normal	13 (6.7) 185 (93.4)	2 (1.01) 196 (99.00)	6.89 (153-30.95)	0.012
Protein S	87.47±25.24	102.46±22.73	0.0001	deficient normal	20 (10.1) 178 (89.9)	3 (1.50) 195 (98.50)	7.30 (2.134-24.996)	0.002
Antithrombin	80.19±30.33	85.46±18.23	0.037	deficient normal	23 (11.6) 175 (88.4)	10 (5.10) 188 (94.90)	2.47 (1.144-5.338)	0.021

VTE: venous thromboembolism; OR (95% CI): odds ratio (95% confidence interval).

Table 4. Frequencies of Factor V Leiden G1691A polymorphism and prothrombin G2021A mutation in all participants (*n* = 396).

	Status	VTE Cases, <i>n</i> (%)	Controls, <i>n</i> (%)	OR (95% CI)	<i>p</i> Value
FVL G1691A	Homozygous (G/G)	190 (96.0)	198 (100.0)	17.89 (1.026-312.150)	0.048
	Heterozygous (G/A)	7 (3.5)	0 (0.0)	15.70 (0.890-276.880)	0.060
	Homozygous (A/A)	1 (0.5)	0 (0.0)	3.05 (0.123-75.220)	0.496
Prothrombin G20210A	Homozygous (G/G)	197 (99.5)	198 (100.0)	0.332 (0.013-08.191)	0.499
	Heterozygous (G/A)	1 (0.5)	0 (0.0)	3.05 (0.123-75.220)	0.496
	Homozygous (A/A)	0 (0.0)	0 (0.0)	0.00 (0.000-0.000)	0.000

FVL G1691A: Factor V Leiden; Homozygous (G/G): homozygous normal genotype; VTE: venous thromboembolism; OR (95% CI): odds ratio (95% confidence interval); Heterozygous (G/A): heterozygous mutant form; Homozygous (A/A): homozygous mutant form.

of the study participants had the homozygous (A/A) mutation (Table 4).

The FVL G1691A mutation was detected in 4.49% of patients ($n = 7/156$) with DVT and in 5.0% ($n = 1/20$) of those with pulmonary thromboembolism (Table 5). Patients with other conditions, such as venous thrombosis of the upper limb, cerebral vein thrombosis, and portal vein thrombosis did not exhibit the FVL G1691A mutation. Similarly, 11 (7.05%) patients with DVT had PC deficiency, one patient had pulmonary thromboembolism, and another patient had portal vein thrombosis. Furthermore, PS deficiency was found in 25.0% ($n = 1/4$) of patients with cerebral vein thrombosis and 10.26% ($n = 16/156$) of patients with DVT, as shown in Table 5. Details regarding AT deficiency in the observed cases are also presented in Table 5.

Table 6 depicts the correlation of DVT, FVL G1691A polymorphism, and PC and PS deficiencies with risk factors, including age, family history, high BMI, contraceptive use and termination. In the study population, DVT was found to be correlated negatively with age ($r = -0.122$, $p < 0.05$) but positively with contraceptive use, high BMI, and history of termination ($p < 0.05$). Age, high BMI, and termination showed negative correlation with the FVL G1691A polymorphism, but these findings were statistically insignificant. Contraceptive use showed a statistically significant ($p < 0.05$) positive correlation with the FVL G1691A polymorphism. Similarly, PC deficiency positively correlated ($r = 0.227$, $p < 0.05$) with high BMI.

The PS deficiency also showed a statistically significant ($p < 0.05$) positive correlation with age and termination.

DISCUSSION

Hemostasis is a delicate balance between procoagulant and anticoagulant factors that maintains blood flow within the blood vessels. Abnormal bleeding tendency or thrombosis occurs when the level of any of the pro coagulant factors and/or anticoagulants is in excess, deficient, or functionally abnormal. Protein C, PS, and AT are the most important natural anticoagulants. Factor V and prothrombin (factor II) are pivotal in the coagulation cascade. Thrombin also plays a crucial role in the conversion of fibrinogen into fibrin and factor XIII into XIIIa to form a stable clot. Simultaneously, thrombin forms a complex with thrombomodulin that activates PC in the presence of PS. The activated PC inhibits the activated coagulation factors V and VIII, thereby limiting the clot formation [11]. Deficiency of PC or PS results in increased susceptibility to thrombosis. Antithrombin inactivates the activated coagulation factors XI, IX, X and II. Single-nucleotide polymorphism of the *FVL* gene results in the development of FVL. It renders FVL resistant to the activated PC, thereby decreasing the anticoagulant function of PC and predisposition to thrombus formation [12]. The prothrombin G20210A mutation of the prothrombin gene exhibits increased synthesis and functional capability of prothrombin, favoring thrombosis

Table 5. Frequencies of factor V Leiden G1691A polymorphism and protein C, protein S and antithrombin deficiencies in various observed clinical features of pregnant women with venous thromboembolism ($n = 198$).

Clinical Features	FVL G1691A, n (%)	PC Deficiency, n (%)	PS Deficiency, n (%)	AT Deficiency, n (%)
Deep vein thrombosis	7/156 (4.49)	11/156 (7.05)	16/156 (10.26)	18/156 (11.54)
Pulmonary thromboembolism	1/20 (5.00)	1/20 (10.00)	1/20 (0.50)	2 (10.00)
Upper limb venous thrombosis	0/10 (0.00)	0/10 (0.00)	2/10 (20.00)	1/10 (10.00)
Cerebral vein thrombosis	0/4 (0.00)	0/4 (0.00)	1/4 (25.00)	1/4 (25.00)
Portal vein thrombosis	0/8 (0.00)	1/8 (12.50)	0/8 (0.00)	1/8 (12.50)

FVL G1691A: Factor V Leiden; PC: protein C, PS: protein S; AT: antithrombin

Table 6. Correlation of deep vein thrombosis, factor V Leiden G1691A polymorphism, and protein C, protein S and antithrombin deficiencies with risk factors among pregnant women ($n = 396$).

Variable Features	Age		Family History		Contraceptives		High BMI		Termination	
	r	p Value	r	p Value	r	p Value	r	p Value	r	p Value
Deep vein thrombosis	-0.122	0.015	0.054	0.286	0.189	0.000	0.042	0.047	0.274	0.000
Factor V Leiden G1691A	-0.033	0.517	0.024	0.635	0.111	0.027	-0.047	0.352	-0.047	0.325
Protein C deficiency	0.036	0.471	-0.053	0.292	0.020	0.693	0.227	0.000	0.036	0.467
Protein S deficiency	0.105	0.035	0.038	0.456	0.059	0.241	0.065	0.197	0.148	0.003
Antithrombin deficiency	-0.110	0.005	0.012	0.751	0.199	0.001	0.126	0.253	0.274	0.001

BMI: body mass index.

[4]. Thus, evaluation of the risk factors, frequency of FVL G1691A, prothrombin G20210A, and deficiency of the most common natural anticoagulants in pregnancy, is essential for better management of patients with VTE and to avoid any untoward clinical manifestations. In this study, approximately 81.3% of the cases exhibited an association with VTE risk factors. The most frequent risk factors associated with VTE were contraceptive use, high BMI, and termination. These findings correlate well with those of previous studies [13-16]. Meanwhile, 10.1% of the cases had a family history of VTE. History of hypertension, surgery, renal diseases, and immobility were comparatively rare clinical manifestations in patients with VTE.

In this study, the frequency of the FVL G1691A heterozygous mutation (G/A) was 3.5%, whereas that of the homozygous mutation (A/A) was 0.5% [OR = 17.71, 95% confidence interval (95% CI) = 1.015-309.05], which is consistent with the results of previous studies [17,18]. The rate of the FVL G1691A polymorphism is almost similar in various pregnancy-associated clinical manifestations [19]. In a group of Sudanese women with preeclampsia, the frequency of such polymorphism was found to be 9.6% [20]. Moreover, only one (0.5%, OR = 0.332, 95% CI: = 0.013-8.191) case was heterozygous (G/A) for prothrombin G20210A polymorphism. These findings are consistent with some studies but in contrast with others. However, a recent study did not detect this mutation in a selected group of pregnant women [20]. In another study conducted in Sudan, the prevalence of prothrombin G20210A mutation was reported as 3.0%, which is higher than that observed in this study [17]. Other researchers have also reported a high prevalence of prothrombin G20210A heterozygous mutations in pregnant women with DVT or PE [19]. By reviewing the ODs, it is evident that although statistically insignificant, patients with VTE were more susceptible to carry the FVL G1691A polymorphism than controls. According to the clinical presentation, of 156 patients with DVT, seven (4.49%) had the heterozygous FVL G1691A (G/A) polymorphism and one of 20 patients with pulmonary thromboembolism had a homozygous (A/A) polymorphism. These data suggest that among the VTE cases, FVL G1691A polymorphism was the most common finding in patients with DVT, which was consistent with other studies that reported a high prevalence of this polymorphism in patients with DVT [16,19,21-24]. Only one case had a heterozygous prothrombin G20210A mutation, representing 0.5% of the study population, in contrast with other studies that reported a slightly high prevalence [19,25]. The results of this study showed that AT deficiency was the most common finding in patients with DVT. The rates of PC and PS deficiencies were slightly lower than the rate of AT deficiency. These findings suggest that AT deficiency is

more commonly associated with DVT than FVL G1691A polymorphism and PC and PS deficiencies among patients with VTE. Similarly, AT deficiency was the most common finding in patients with PE and venous thrombosis of the upper limb. The correlation analysis disclosed a statistically significant ($p < 0.05$) association with age ($r = -0.122$), contraceptive use ($r = 0.189$), and termination ($r = 0.274$). The FVL G1691A polymorphism and PC and PS deficiencies also demonstrated strong correlation with contraceptive use, high BMI, age and termination. These findings are consistent with those of previous studies [4-6].

In conclusion, among the VTE cases, FVL G1691A polymorphism and PC, PS, and AT deficiencies were the most common findings in patients presenting with DVT. The AT deficiency was more common in the study population than PC and PS deficiencies. Contraceptive use, high BMI, and termination correlated strongly with FVL G1691A polymorphism and PC and PS deficiencies in patients with VTE. Major limitation of the study was no follow-up of the pregnant patients with VTE to observe the effect of FVL G1691A polymorphism and low levels of PC, PS and AT on the outcome of pregnancy. We intend to conduct further studies to explore the role of these factors in the pregnancy outcome.

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