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ORIGINAL ARTICLE

Clinical characteristics and thrombophilia associated gene variants in Egyptians with unprovoked venous thromboembolism: three centers experience

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Abstract

Background Thrombophilias are characterized by excessive venous and arterial thrombosis at regular or unusual sites. It may result from inherited, acquired, or a combination. Hereditary thrombophilia (HT) is detected in 30–40% of patients with thromboembolism. Venous/arterial thrombosis is considered a multifactorial disorder, some patients may have more than one risk factor which may be transient or permanent.

Objectives Assess the clinical characteristics of patients with unprovoked thromboembolic events and the role of inherited thrombophilia as a causative or additive risk factor.

Methods 210 consecutive adult patients with unprovoked thromboembolic events were reviewed in hematology units at three tertiary Egyptian centers between September 2022 and September 2023. The diagnosis of thromboembolic events was confirmed by clinical and radiological findings. Laboratory screening for thrombophilia-associated.

Results Among our patients, 53(25.2%) patients presented with isolated DVT, followed by portal vein thrombosis, 32(15.2%) had a pulmonary embolism, and sagittal sinus thrombosis was developed in 23(10.9%) patients.

Conclusion Younger people who experience spontaneous thromboembolism run the chance of having hereditary thrombophilia; the more mutations discovered, the higher the risk of thrombosis; the lower leg and deep vein thrombosis were the most common sites. Lastly, MTHFR C677T was the most common polymorphism in Egyptians, detected in almost half of the cases.

Keywords Thrombophilia · Inherited · Thromboembolism · Deep venous thrombosis · Factor V Leiden

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Introduction

Thrombophilia is a term used to describe a group of diseases in which there is a predisposition to excessive venous and /or arterial thrombosis at usual or uncommon sites, which carries a significant source of morbidity and mortality. Venous/arterial thrombosis is considered a multifactorial disorder, some patients may have more than one risk factor which may be transient or permanent. These factors can be inherited, acquired or combined [1].

Common acquired risk factors include major surgeries, recent hospital admission with immobility, pregnancy or infections such as COVID-19. A family history of higher thrombotic events or a proven genetic mutation, such as factor V Leiden (FVL), protein C and S deficiencies, antithrombin deficiency, prothrombin (PT) 20,210 A mutations, methylenetetrahydrofolate reductase (MTHFR) C677T causing hyper-homocysteinemia, or plasminogen activator inhibitor-1 (PAI-1), are examples of hereditary or genetic causes of thrombophilia [1, 2].

Hereditary thrombophilia (HT) is detected in at least 30–40% of patients with thromboembolism, despite of conflicting findings regarding its incidence in these patients. There are differences in the clinical presentation. Before turning 40, some patients have recurrent, unprovoked thromboembolic episodes, whereas others have no symptoms at all [3, 4].

The available tests for HT are sensitive but expensive, and time-consuming and their role in the management of unprovoked thromboembolism remains poorly defined. Although HT may increase the risk of both provoked and unprovoked thromboembolism, it remains unclear whether the presence of hereditary factors significantly affects the clinical decision of lifelong anticoagulation or modifies the risk of recurrence [5, 6].

Aim of the study

In this study, we aimed to assess the clinical characteristics of adult patients presented with unprovoked thromboembolic events and evaluate the incidence, and the role of inherited thrombophilia as a causative or additive risk factor.

Patients and methods

In this cross-sectional multicenter study, a total of 210 consecutive adult patients with unprovoked thromboembolic events were reviewed in hematology units at three tertiary Egyptian centers between September 2022 and September 2023.

Exclusion criteria

- Patients with inadequate clinical or laboratory data.
- Patients with any of the provoking factors for thromboembolism as recent surgery, trauma, sepsis, immobilization for at least 7 days, females on oral contraceptive pills, hormonal replacement therapy, pregnancy, or in the puerperium and those with a known cardiac or vascular cause of thrombotic events (and we did echocardiogram, also carotid media intimal thickness was assessed also by ultrasound to exclude cardiac or diabetic effect of thrombophilia).
- Patients with active cancer within 6 weeks to 3 months before the first thromboembolic event and patients with underlying advanced liver disease or acquired thrombophilia factors such as myeloproliferative neoplasms or antiphospholipid syndrome.

Detailed history has been reviewed including data about age, sex, associated comorbidities, and family history of any thromboembolic events, we recorded each patient's previous history of thromboembolism, date, the site initially presented symptoms, and presence of other predisposing factors for a thrombotic event, patients were treated according to the standard protocol; they were initially treated with low molecular weight heparin and then shifted to warfarin or direct oral anticoagulant drugs for at least 3 months according to their thrombophilia risk.

The diagnosis of thromboembolic events was confirmed by both clinical findings and imaging using multi-detectable computed tomography (CT) scans, angiogram, or duplex ultrasonography according to the suspicious site of thrombosis either pulmonary embolism (PE), deep venous thrombosis (DVT) of extremities, portal, splanchnic, retinal (considering controversies in HT) or cerebral veins thrombosis.

Data collection

- Informed consent was provided by all patients authorizing the use of their data for research purposes after an explanation of the benefits and risks of the study. Blood samples were obtained under complete a septic technique before the beginning of anticoagulant therapy. All precautions will be taken to avoid any infections or hazards during sampling or imaging. The study was approved by the local ethical and scientific committee of Tanta University, ethical approval number (36256) and approved by Benha University, and Menoufia University, Egypt, dated 2023.

- Detailed history has been reviewed including data about age, sex, associated comorbidities, and family history of any thromboembolic events. We recorded each patient's previous history of thromboembolism. Date, site, initially presented symptoms, and presence of other predisposing factors for thrombotic event. Patients were treated according to the standard protocol; they were initially treated with low molecular weight heparin and then shifted to warfarin or direct oral anticoagulant drugs for at least 3 months according to their thrombophilia risk.

Laboratory investigations

1. Standard laboratory tests including complete blood counts, prothrombin time, activated partial thromboplastin time, and evaluations of the liver and kidneys were performed on each patient.
2. Laboratory testing for polymorphisms linked to thrombophilia, such as factor V Leiden, prothrombin gene mutation G20210A, protein C, protein S, antithrombin III, factor XIII V34 L, b-fibrinogen-455G > A, plasminogen activator inhibitor-1 (PAI-1) 4G/5G, methylene tetrahydrofolate reductase (MTHFR) C677 T, MTHFR A1298C, angiotensin-converting enzyme (ACE) I/D, apolipoprotein B R3500Q (Apo B), and apolipoprotein E (Apo E).

- The chromogenic method was used for the quantitative assay of protein S, C, and antithrombin III, STA-R Evolution was used to determine the activity level of protein C, S by using specific kits, i.e., protein C-STAGO, STA ATIII STAGO, and protein S-STAGO, respectively.
- The genotyping assays of Factor V Leiden, prothrombin G20210A, and MTHFR are based on FRET (Fluorescence Resonance Energy Transfer)-allowing distinction between the three genotypes: homozygous wild type, homozygous mutant, or heterozygous, The extracted and purified DNA was analyzed by Real-Time PCR genotyping for these thrombophilic variants, commercial kits (Roche Diagnostics), the isolated DNA, DNA (genomic DNA) was isolated from 210 µl peripheral blood using the MagNA pure LC DNA Isolation kit and the automated extractor based on the magnetic-bead technology (MagNA pure LC Instrument).

Table 1 Socio-demographic criteria among the studied groups

	The studied groups $N=210$
Age (years)	30.87 ± 8.48
Mean ± SD	16–49
Range	
Age groups	32 (15.2)
≤ 20 years	151 (71.9)
21–40 years	27 (12.9)
> 40 years	
Sex	110 (52.4)
Male	100 (47.6)
Female	
Family history	82 (39.0)
Positive	128 (61.0)
Negative	
Co-morbidity	178 (84.8)
Negative	16 (7.6)
HTN	10 (4.8)
DM	3 (1.4)
HCV (non cirrhotic)	3 (1.4)
Hypothyroidism	

HTN = Hypertension. DM = Diabetes Miletus. HCV = Hepatitis C Virus.

Statistical analysis

Data were transferred to a personal computer, classified, and analyzed using SPSS (version 20, SPSS Inc., Illinois, Chicago, USA). The quantitative data was described as mean, standard deviation, and range while qualitative data was described as number and percentage. Z test was used to compare percentages presenting symptoms among sex, age groups, and family history subcategories and the chi-square test was used to compare qualitative variables in different types of thromboembolic disorders while quantitative data (normally distributed and not normally distributed) were compared using ANOVA and Kruskal Wallis tests respectively. P-value < 0.05 was considered statistically significant.

Results

In this multicenter study, the clinical and laboratory data of a total of 210 adult patients who developed unprovoked thromboembolic events between March 2022 and March 2023 were reviewed.

Baseline characteristics of the study participants

The patient's baseline characteristics are described in (Table 1). The age ranged from 16 to 49 years with a mean age at the time of the thrombotic event of 30.87 ± 8.48, the majority of patients aged between 21 and 40 years. Among our patients, males represented 52.4% and females 47.6%. 82 (39%) patients had a positive family history of thrombosis. Thirty-two patients were reported to have comorbidities which included diabetes, hypertension, hypothyroidism, and chronic hepatitis C infection (HCV).

Table 2 The presenting symptoms in relation to sex among the studied patients

The presenting symptom	The studied groups $N=210$	Sex		Z test	*P* value
		Male $N=110$	Female $N=100$		
DVT	53 (25.2)	31 (58.5)	22 (41.5)	1.55	0.12
Portal vein thrombosis	33 (15.7)	17 (51.5)	16 (48.5)	0.0	1.0
Pulmonary embolism	32 (15.2)	18 (56.2)	14 (43.8)	0.75	0.45
Saggital sinus thrombosis	23 (11.0)	10 (43.5)	13 (56.5)	0.59	0.55
Mesenteric vascular occlusion	17 (8.1)	12 (70.6)	5 (29.4)	2.06	0.04
Retinal vein thrombosis	20 (9.5)	10 (50.0)	10 (50.0)	0.32	0.75
Cerebral sinus thrombosis	6 (2.9)	2 (33.3)	4 (66.7)	0.58	0.56
Ischemic stroke	10 (4.8)	4 (40.0)	6 (60.0)	0.45	0.65
Recurrent peripheral thrombophlebitis	4 (1.9)	2 (50.0)	2 (50.0)	0.71	0.48
DVT & pulmonary thrombosis or portal VT	12 (5.7)	4 (33.3)	8 (66.7)	1.22	0.22

Table 3 The presenting symptoms in relation to age groups and family history among the studied patients

The presenting symptom	Age groups			Test	*P* value	Family history		Test	*P* value
	≤20	21–40	>40			Positive $N=110$	Negative $N=100$		
DVT	10 (18.9)	37 (69.8)	6 (11.3)	5.93	<0.001	16 (30.2)	37 (69.8)	3.89	<0.001
Portal vein thrombosis	4 (12.1)	25 (75.8)	4 (12.1)	4.96	<0.001	16 (46.5)	17 (51.5)	0.0	1.0
Pulmonary embolism	0 (0.0)	31 (96.9)	1 (3.1)	7.5	<0.001	14 (43.8)	18 (56.2)	0.75	0.45
Saggital sinus thrombosis	10 (43.5)	13 (56.5)	0 (0.0)	3.93	<0.001	10 (43.5)	13 (56.5)	0.59	0.55
Mesenteric vascular occlusion	4 (23.5)	7 (41.2)	6 (35.3)	0.73	0.47	6 (35.3)	11 (64.7)	1.37	0.17
Retinal vein thrombosis	4 (20.0)	12 (60.0)	4 (20.0)	2.26	0.02	8 (40.0)	12 (60.0)	0.95	0.34
Cerebral sinus thrombosis	0 (0.0)	6 (100)	0 (0.0)	2.89	0.004	2 (33.3)	4 (66.7)	0.58	0.56
Ischemic stroke	0 (0.0)	8 (80.0)	2 (20.0)	3.20	0.001	2 (20.0)	8 (80.0)	2.24	0.02
Recurrent peripheral thrombophlebitis	0 (0.0)	4 (100)	0 (0.0)	2.12	0.03	2 (50.0)	2 (50.0)	0.71	0.48
DVT & pulmonary thrombosis or portal VT	0 (0.0)	8 (66.7)	4 (33.3)	3.03	0.002	6 (50.0)	6 (50.0)	0.41	0.68

The clinical presentation of patients

The clinical characteristics of patients, the site of the thrombotic event, and its relation to the patient's age, sex, and family history were summarized in (Table 2, and 3).

Of the 210 patients, 53 (25.2%) showed isolated DVT. Furthermore, 33 (15.7%) had portal vein thrombosis, 32 (15.2%) had a pulmonary embolism, 23 (11%) developed sagittal sinus thrombosis, 17 (8.1%) experienced mesenteric vascular occlusion, 20 (9.5%) were diagnosed with retinal vein thrombosis, 6 (2.9%) had cerebral sinus thrombosis, 10 (4.8%) had ischemic stroke, and 4 (1.9%) had recurrent peripheral thrombophlebitis. Additionally, 12 (5.7%) patients presented with DVT in combination with either pulmonary or portal vein thrombosis.

We examined the effect of patient factors on the incidence of different types of thrombotic events. The incidence of DVT, portal vein thrombosis, pulmonary embolism, and mesenteric vascular occlusion were higher in males while females had a higher incidence of sagittal sinus thrombosis, ischemic stroke, and cerebral sinus thrombosis. However, no significant difference could be detected between males and females except for mesenteric vascular occlusion which was significantly higher in males (70.6%) versus (29.4%) in females.

A significantly higher incidence of all types of thrombotic events was found in patients in the age group (21–49) years, except in those presented with mesenteric vascular occlusion. A positive family history was not identified as a risk factor for thrombosis among our study participants. A significantly higher incidence of DVT and recurrent peripheral thrombophlebitis was found in patients with a negative family history of thrombosis (69.8%versus 30.2% and 80% versus 20%), respectively.

Thrombophilia tests among study participants

The MTHFR C677T was the most frequent genetic cause of VTE, followed by the FV Leiden and MTHFR A1298C mutations. The MTHFR C677T mutation was found positive in 55.2% of patients and 44.8% of them had a heterozygous pattern, while the MTHFR A1298C mutation was found in 39.5% of patients and the majority of them had a heterozygous mutation (26.7%). We identified 111(52.9%) patients with mutated FV Leiden, (17.6% of them were homozygous and 35.2% were heterozygous). Twenty-one

(10%) patients had mutated B fibrinogen and heterozygous and homozygous mutations of prothrombin gene G20210A were detected in 15.7% and 1% of patients; respectively. Only 6.7% of patients presented with heterozygous Factor XIII V34L.The majority of our patients had no mutations in ACDI/D, APO B, and APO E. The PAI-1 4G/5G polymorphism was not identified in any patient (Table 4).

The values of measured markers, the presence of combined thrombophilia gene mutations, and their relation to the development of different thrombo-embolic disorders were demonstrated in (Table 4). Protein C showed a difference between thromboembolic disorders with the highest value in DVT (110.1 ± 30.5) and retinal vein thrombosis (103.1 ± 9.3) and the lowest values in cerebral sinus thrombosis and mesenteric vascular occlusion. While Protein S showed a non-significant difference between different thromboembolic disorders. Antithrombin III was significantly higher in DVT (107.9 ± 20.9) and portal vein thrombosis (107.7 ± 39.8) and lowest values in pulmonary embolism (86.5 ± 36.4). Factor V Leiden was mutated in all patients who had recurrent peripheral thrombophlebitis (100%), DVT (75%), and those with sagittal sinus thrombosis only 13% had mutations.

MTHFR C677T was mutated in 55.2% (7.6% homogenous & 47.6% heterogenous), and most of the mutated patients have DVT portal vein thrombosis, pulmonary embolism, sagittal vein, and retinal vein thrombosis. MTHFR A1298C was mutated in 83 patients (39.5%). Most of them developed DVT and portal vein thrombosis, and the lowest events were sagittal vein thrombosis and ischemic stroke. All patients with recurrent peripheral thrombophlebitis (100%) had heterogeneous mutations.

Beta fibrinogen was mutated in only 21 patients (10%), including 8 patients with Sagittal sinus thrombosis 6 patients with DVT, (and 3 and 4) patients with the portal vein and mesenteric venous thrombosis respectively while those presented with retinal vein thrombosis, cerebral sinus thrombosis, recurrent peripheral thrombophlebitis, and combined thrombosis had no mutations in Beta fibrinogen.

Prothrombin G20210A was abnormally mutated in 35 (16.7%) of total patients, including 7 patients with DVT, 11 patients with portal vein thrombosis, and also heterogeneous mutation in all patients presented with recurrent thrombophlebitis (4 patients). So, those patients have combined heterogeneous mutation of factor V Leiden and prothrombin gene mutation 2021.

Factor XIIIV34L heterogenous mutations were only found in 14 patients including pulmonary embolism, sagittal sinus and retinal vein thrombosis. ACE I/D, Apo B, and APO E showed a very low level of abnormality (< 5%), and PAI-1 4G/5G had 100% normal.

Discussion

Venous thromboembolism (VTE) is a major global health issue that impairs patient's quality of life QoL. The disease is complex, meaning that environmental, acquired, and inherited risk factors interact to cause it. Genetic mutations' impact on hemostasis and the elevated risk of intravascular coagulation have been covered in earlier research, indicating that inherited thrombophilia heightens the vulnerability to thromboembolic events [7]. Numerous studies back up the identification of HT patients as a means of reducing morbidity and mortality, particularly in young, productive people [8]. In the current multicenter study in the delta region of Egypt, we examined the incidence of inherited thrombophilia and its potential correlation with the risk of unprovoked thrombosis in 210 patients by assessing clinical presentation, patient characteristics, and the existence of additional risk factors.

In our study, the majority of patients had one or more HT risks. More men than women were afflicted, and 71.9% of patients who reported spontaneous unprovoked thrombotic episodes were under 40 years old. Interestingly, however, none of the patients had a strong family history of thrombosis. The most frequent events were DVT, which was followed by portal vein thrombosis, pulmonary embolism, and mesenteric thrombosis. Males were much more likely than females to experience mesenteric vein thrombosis but cerebral events were more common in women.

The most common subtypes were MTHFR C677T, FV Leiden, and MTHFR A1298C, with PG 20,210 A coming in second. No patient in our study had the PAI-1 4G/5G mutation. Of the patients who presented with DVT, CVT, and ischemic stroke, we did not find any with mutations in factor XIIIV34L, ACE I/D, APO E, APO B, or PAI-4G.

Numerous earlier research that examined the clinical features of HT found that younger individuals with a family history had a higher risk of spontaneous thrombosis [9, 10]. In a Korean study by Lee SY et al., they also reported that patients in the HT group had a male predominance and were significantly younger than 45 years. Consistent with our findings, DVT was the most common thrombotic event in patients who were lower extremities. In contrast to our findings, they discovered that their HT group had a higher frequency of family history of VTE [11].

After combining our data, it demonstrated that FV Leiden, MTHFR C, MTHFR A, and PT G20210A variants were substantially more common in DVT patients. which are consistent with results of a previously published study [12]. Our findings in this regard are also supported by reports by Meyer et al. and Arsov et al. Unfortunately, we did not measure the serum homocysteine level to link its elevated role in MTHFR gene polymorphism as they did [13, 14].

Table 4 Laboratory investigations among the studied group

	The studied group $N=210$	DVT $N=53$	Portal vein thrombosis $N=33$	Pulmonary embolism $N=32$	Saggital sinus thrombosis $N=23$	Mesenteric vascular occlusion $N=17$	Retinal vein thrombosis $N=20$	Cerebral sinus thrombosis $N=6$	Ischemic stroke $N=10$	Recurrent peripheral thrombophlebitis $N=4$	DVT & pulmonary thrombosis or portal VT ($N=12$)	*P* value
Protein C	98.72 ± 30.86	110.1 ± 30.5	104.2 ± 41.2	88.0 ± 35.2	92.0 ± 21.6	84.9 ± 32.4	103.1 ± 9.3	84.3 ± 30.5	91.8 ± 22.7	88.0 ± 0.0	103.8 ± 11.0	0.02
Mean ± SD	21–200	47 – 160	40–200	21–128	55–115	45–120	85–120	45–104	50–112	88–88	89–112	
Range												
Protein S	96.28 ± 28.55	95.2 ± 23.6	98.8 ± 34.9	86.3 ± 37.7	106.7 ± 31.3	88.6 ± 28.5	100.9 ± 15.0	88.7 ± 35.1	105.4 ± 17.7	89.0 ± 0.0	102.3 ± 11.3	0.31
Mean ± SD	29–160	29–143	44–150	30–150	65–160	55–132	80–120	66–134	87–130	89–89	88–124	
Range												
AT- III	100.55 ± 27.45	107.9 ± 20.9	107.7 ± 39.8	86.5 ± 36.4	90.7 ± 18.4	99.5 ± 20.1	106.0 ± 9.4	104.7 ± 3.6	100.4 ± 34.5	99.0 ± 0.0	95.8 ± 15.0	0.03
Mean ± SD	29–202	62–143	55–202	29–137	45–111	54–125	88–118	100–107	37–132	99–99	78–119	
Range												
Factor V Leiden	99 (47.1)	13 (24.5)	17 (51.5)	14 (43.8)	20 (87.0)	9 (52.9)	12 (60.0)	4 (66.7)	6 (60.0)	0 (0.0)	3 (33.3)	< 0.001
Normal	37 (17.6)	10 (18.9)	4 (12.1)	14 (43.8)	1 (4.3)	4 (23.5)	0 (0.0)	0 (0.0)	2 (20.0)	0 (0.0)	2 (17.6)	
P. homozygous	74 (35.2)	30 (56.6)	12 (36.4)	4 (12.5)	2 (8.7)	4 (23.5)	8 (40.0)	3 (33.3)	2 (20.0)	4 (100)	6 (50.0)	
P. heterozygous												
MTHFR C677T	94 (44.8)	25 (47.2)	8 (24.2)	16 (50.0)	8 (34.8)	14 (82.4)	6 (30.0)	2 (33.3)	6 (60.0)	3 (75.0)	6 (50.0)	< 0.001
Normal	16 (7.6)	0 (0.0)	9 (27.3)	4 (12.5)	0 (0.0)	0 (0.0)	2 (10.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	
P. homozygous	100 (47.6)	28 (52.8)	16 (48.5)	12 (37.5)	15 (65.2)	3 (17.6)	12 (60.0)	4 (66.7)	4 (40.0)	0 (0.0)	6 (50.0)	
P. heterozygous												
MTHFR A1298C	127 (60.5)	41 (77.4)	21 (63.6)	25 (78.1)	2 (8.7)	12 (70.6)	12 (60.0)	2 (33.3)	8 (80.0)	0 (0.0)	4 (33.3)	< 0.001
Normal	27 (12.9)	2 (3.8)	2 (6.1)	3 (9.4)	2 (8.7)	0 (0.0)	4 (20.0)	0 (0.0)	2 (20.0)	4 (100)	8 (66.7)	
P. homozygous	56 (26.7)	10 (18.9)	10 (30.3)	4 (12.5)	1 (82.6)	5 (29.4)	4 (20.0)	4 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												
B- fibrinogen	189 (90.0)	47 (88.7)	30 (90.9)	32 (100)	15 (65.2)	13 (76.5)	20 (100)	6 (100)	10 (100)	4 (100)	12 (100)	< 0.001
Normal	5 (2.4)	0 (0.0)	1 (3.0)	0 (0.0)	0 (0.0)	4 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. homozygous	16 (7.6)	6 (11.3)	2 (6.1)	0 (0.0)	8 (34.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												
Prothrombin	175 (83.3)	44 (83.0)	22 (66.7)	29 (90.6)	23 (100)	13 (76.5)	16 (80.0)	6 (100)	10 (100)	0 (0.0)	12 (100)	< 0.001
G20210A	2 (1.0)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Normal	33 (15.7)	7 (13.2)	11 (33.3)	3 (9.4)	0 (0.0)	4 (23.5)	4 (20.0)	0 (0.0)	0 (0.0)	4 (100)	0 (0.0)	
P. homozygous												
P. heterozygous												
Factor X||| V34L	196 (93.3)	53 (100)	33 (100)	24 (75.0)	19 (82.6)	17 (100)	18 (90.0)	6 (100)	10 (100)	4 (100)	12 (100)	< 0.001
Normal	14 (6.7)	0 (0.0)	0 (0.0)	8 (25.0)	4 (17.4)	0 (0.0)	2 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												
ACE I/D	201 (95.7)	53 (100)	29 (87.9)	32 (100)	22 (95.7)	13 (76.5)	20 (100)	6 (100)	10 (100)	4 (100)	12 (100)	0.002
Normal	9 (4.3)	0 (0.0)	4 (12.1)	0 (0.0)	1 (4.3)	4 (23.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												
APO B	208 (99.0)	53 (100)	31 (93.9)	32 (100)	23 (100)	17 (100)	20 (100)	6 (100)	10 (10.0)	4 (100)	12 (100)	0.29
Normal	2 (1.0)	0 (0.0)	2 (6.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												

Table 4 (continued)

	The studied group $N=210$	DVT $N=53$	Portal vein thrombosis $N=33$	Pulmonary embolism $N=32$	Saggital sinus thrombosis $N=23$	Mesenteric vascular occlusion $N=17$	Retinal vein thrombosis $N=20$	Cerebral sinus thrombosis $N=6$	Ischemic stroke $N=10$	Recurrent peripheral thrombophlebitis $N=4$	DVT & pulmonary thrombosis or portal VT ($N=12$)	*P* value
APO E	205 (97.6)	53 (100)	29 (87.9)	32 (100)	22 (95.7)	17 (100)	20 (100)	6 (100)	10 (10.0)	4 (100)	12 (100)	0.04
Normal	2 (2.4)	0 (0.0)	4 (12.1)	0 (0.0)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												
PAI-1 4G/5G	210 (100)	53 (100)	33 (100)	32 (100)	23 (100)	17 (100)	20 (100)	6 (100)	10 (10.0)	4 (100)	12 (100)	------
Normal	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
P. heterozygous												

AT-III = antithrombin III,

Recent multicenter research involving 528 individuals found that 28% of the included patients had positive HT test results. The most common mutation was FV Leiden, which was followed in importance by PT G20210A mutations. It was observed that neither patients nor multiple concurrent HT [15] were compound heterozygotes for FV Leiden and PT G20210A mutations, which contradicted our findings as many of our patients tested positive for compound mutations.

We found that, in contrast to our findings, a recent study by Obaid et al. examined the incidence of HT in 227 consecutive patients with pulmonary embolism who had at least one marker positive for HT. The majority of his patients had protein S, protein C, antithrombin III deficiency, and hyperhomocysteinemia, while a small minority had FV Leiden [16].

Recently, Idris A, who studied the prevalence of three thrombophilic genes in twenty Egyptian patients from one center, found that the highest genotyping frequency among his patients was FVL G1691A polymorphism (72.7.0%) followed by MTHFRC677T polymorphism in 45% and the lowest frequency was F2 G20210A polymorphism [17].

Patients with DVT had a lower incidence of polymorphism than those with portal vein thrombosis, according to the results of our study's examination of PT G20210A polymorphism. On the other hand, according to certain writers, DVT patients had a higher frequency of PT G20210A polymorphism than controls [18], which was also the case in research by Lijfering et al. The SNPs PT G20210A and FV Leiden did not affect the risk of venous thrombosis [19].

Thirty-nine (18.6%) of our patients experienced cerebral thrombotic events, with the majority showing a high incidence of MTHFR C677T. this meet with a recent study by El-Khawaga et al., which examined the link between genetic polymorphisms in MTHFR and susceptibility to stroke in the Egyptian population, reported a high prevalence of MTHFR mutations in patients with ischemic stroke [20]. In contrast to our findings, a recent investigation in Saudi Arabia revealed that protein S deficiency was the most commonly identified factor contributing to cerebral venous sinus thrombosis, accounting for 3.6% of all strokes caused by this condition. In our patients to rule out any cardiac or diabetic effects for causing thrombophilia we did an echocardiogram and assessed carotid intima-media thickness via ultrasound in [21].

Among the unprovoked patients, protein S and protein C insufficiency were the most prevalent HT, according to another retrospective multicenter investigation [22].

Prior research discouraged HT testing in patients who had risk indicators that were triggered. Fascinatingly, 16 out of 29 individuals in a recent study by Elbadry et al. assessing the prevalence of HT in non-critically ill COVID-19

patients with numerous rare thromboembolic episodes had underlying genetic reasons for thrombophilia identified. Among their patients, the most common hereditary thrombophilias were PGM-G20210A, Protein C, and Antithrombin-III deficits [23].

The study by Alkhiary et al. compared the occurrence of thrombophilia gene polymorphisms in Egyptians with those from different ethnic backgrounds. They found a higher prevalence of FV Leiden in Egypt. In addition, the prevalence of the MTHFR 677T allele among Egyptian patients was high. The prevalence of MTHFR 1298 C was similar to other countries such as Spain, Italy, Lebanon, and Cyprus. The prevalence of the FGB-455 A allele in healthy Egyptians is similar to that in the other countries except for China. The prevalence of PAI-1-675 4G is high, similar to all except Africa, where it is low. The prevalence of the GPIIIa 1565T allele in Egypt is similar to that in Lebanon and Greece [24].

According to numerous guidelines, patients with VTE should be divided into two groups: those at unprovoked or provoked risk of recurrence, and the unprovoked/high-risk group should be kept together unless there is a known risk of bleeding [25, 26].

The most recent ASH recommendation for thrombophilia testing, however, states that although indefinite antithrombotic medication is advised for the majority of patients with recurrent unprovoked VTE, most individuals do not require routine thrombophilia testing. Although indefinite anticoagulation is advised for those with thrombophilia, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. Testing may be considered in younger patients with nonsurgical transient factors, a strong family history of high-risk HT, and patients with cerebral or splanchnic venous thrombosis who have completed primary treatment in a setting where anticoagulation would be discontinued [27].

The British Society of Haematology recommends genetic testing to find the causative variants causing phenotypically identified deficiencies of AT, PC, and PS when the results will impact management because patients with hereditary natural anticoagulant deficiency have a 15-fold increased risk of VTE [28].

Limitations of the study

The present study has many limitations that should be mentioned. We did not follow up on patients presented with the first episode and we may have missed recurrent events that occurred afterwards, this may be explained by the study period which was only one year. The study applied to populations with similar ethnic backgrounds. The number of patients with each specific type of HT was relatively small; thus meaningful comparisons could not be made between specific types of HT Despite these limitations, we had a relatively homogenous group of patients diagnosed with one or more inherited thrombophilia presented with different types of unprovoked thrombosis, were diagnosed and initially managed at three Egyptian centers using the standard protocols. Our data might provide the basis for further larger prospective studies with longer follow-up periods and different ethnic populations aiming at optimizing hereditary and acquired thrombophilia testing in patients with and without provoking risks. Finally, we did not do homocysteine blood level.

Conclusion

Unprovoked thromboembolism in younger has the risk of being associated with inherited thrombophilia, the more mutations found the more the risk for thrombosis the most prevalent site was the lower limp and the deep venous thrombosis. Finally, the most prevalent mutation in Egyptians was MTHFR C677T which was found in more than half of patients.

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Author contributions Alaa Efat and Mona Mahrous wrote the manuscript and analyzed the data. Medhat Elamawy and Hiam Eleleimy with the previous two authors performed data collection and manuscript preparation. Suzan Elmorshdy performed the lab studies and analyses. Reda Abdellatif did the statistical analysis. Sabry Shoeib and Abdelmoneiem Ahmed were responsible for the selection and follow-up of patients. All authors revised the study and reviewed the article.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval The study was approved by the local ethical and scientific committee of Tanta University, Benha University, and Menoufia University, Egypt, dated 2023. Under number (36256).
All subjects agreed to participate in this study with consent obtained.
All subjects agreed to share and publish this study with consent obtained.

Competing interests The authors declare no competing interests.

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