Hypercoagulable State

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Abstract

Background : The hypercoagulable state results from a complex interplay of blood coagulation factors, coagulation-inhibitory factors, platelets and the vascular endothelium. Imbalance of the complex interplay between these factors results in thrombosis often complicated by embolism. The causes of thrombosis are varied and maybe congenital or acquired. The current interest is centered on the congenital deficiency of coagulation inhibitors as there is an increasing awareness of their involvement in thrombosis, especially in the young.

Methods: A total of 42 patients with thrombosis were studied. The most common clinical presentation was deep vein thrombosis. All the cases were evaluated for coagulation inhibitors Antithrombin, resistance to activated protein C, Protein C and Protein S using standard assay kits.

Results: Resistance to activated protein C (n=10) was seen to be the commonest cause of thrombophilia. This was followed by deficiency of Antithrombin (n=4), Protein C (n=3) and Protein S (n=2). Majority of our cases were in the third decade of life.

Conclusion: The identification of the underlying aetiology is important for instituting specific therapy and patient management.

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Key Words : Thrombophilia; Coagulation inhibitors

Introduction

Patients presenting with a wide clinical spectrum of sequelae of thrombosis need investigation of the underlying hypercoagulable state. These patients, especially if young, need further evaluation if the thrombosis is complicated by embolism at unusual sites. This study is an in-depth analysis of the cause of thrombophilia. Congenital deficiency of coagulation inhibitors Antithrombin, Protein C, Protein S and resistance to activated Protein C has been studied in this cohort of 42 patients.

Materials and Method

42 patients of thrombosis diagnosed over a period of 2 ½ years formed the basis of this prospective study for evaluation of the hypercoagulable state. All patients had been diagnosed by radioimaging techniques for presence of thromboembolism. The history of associated systemic disorder, family history of thrombosis and intake of oral contraceptive pills (OCP) in female cases was noted.

Investigations included blood counts, peripheral blood smear, urine study for haemoglobinuria, lipid profile, liver function tests including serum proteins, blood urea and serum creatinine, Ham's test and sucrose lysis test for PNH.

All patients were subjected to a screening coagulogram which included prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and plasma

fibrinogen [1,2]. To exclude the presence of lupus anticoagulant (LAC), tests like APTT – LA and Kaolin clotting time (KCT) were performed [3]. Anti cardiolipin antibodies for both IgG and IgM were done to rule out antiphospholipid antibody (APLA) syndrome [4]. The above tests excluded hyperlipidaemias, hyper-gammaglobinaemias, dysfibrinogenemias, PNH, Nephrotic Syndrome and the APLA syndrome.

After exclusion of common etiologies of thrombosis, investigations were performed to assay the levels of Protein C, Protein S, Anti-Thrombin and resistance to activated Protein C. Once, the PPP was prepared, the screening coagulogram and tests for lupus anticoagulant and anti-cardiolipin antibodies were performed. After these samples for Protein C, Protein S, Anti-Thrombin and activated Protein C resistance assays were stored in the deep freezer at –70°C in multiple aliquots. Estimation of Protein C, Protein S and APCR were performed using kits from STAGO, France on the STAGO, ST-4 semi automated coagulation analyser as per the manufacturers instructions. It was ensured that at the time of sample collection, no patient was on Herapin or oral anticoagulation therapy.

25 age and sex matched controls were also subjected for Protein C, Protein S and APCR studies. Reference values used in the study (Mean ±2SD) were

Protein C assay	70 – 140%
Protein S assay	65 – 140%

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Antithrombin assay	80 – 120%
APC-R	> 120 secs

Results

Initially, 54 patients were investigated for thrombophilia. Six were positive for APLA and excluded. One, with Cerebral Vein Thrombosis (CVT) had PNH. Five were lost to follow-up. The balance of 42 were evaluated.

There were 22 cases, who presented with DVT, 13 male and 9 female, the youngest was a 14 year old female, while the oldest was a 73 year old male (Tables 1 & 2). Other forms of thrombo-embolic disorders (n=20) studied are shown in Table 3. Six patients had recurrent first to mid-trimester foetal loss. Of these, we found one case each of APC-resistance and Protein C deficiency. Four patients each of stroke in young and vaso-occlusive coronary artery disease in young were also evaluated. All four with coronary artery disease manifested as acute MI, and had coagulation abnormalities.

Table 1
Spectrum of clinical diagnosis

Diagnosis	No. of patients
• Deep vein thrombosis	22
• Stroke in young	04
• Vaso-occlusive coronary artery disease in young	04
• Hepatic and portal vein thrombosis	02
• Pulmonary thrombo-embolism	01
• Nephrotic syndrome	02
• Primary pulmonary hypertension	01
• Recurrent foetal loss	06
Total	42

Table 2
Deep vein thrombosis (n=22)

S.No.	Patient	Age (yrs)	Sex	Associated conditions	Protein C %	Protein S %	AT %	APC-R (sec)
1	KC	62	M	-	103.9	86.4	94.6	96 sec (+ve)
2	RS	34	M	Pul. embolism	107.5	85.6	61	128
3	SS	68	F	Ca rectum	112.5	78.6	88	108 sec (+ve)
4	RL	14	F	-	115.0	115.5	82	98 sec (+ve)
5	RN	43	M	Pul. embolism	48.9	88.6	110.0	132
6	SA	36	M	Trans myelitis	130	86.0	100.6	122
7	SS	26	F	-	84.6	79.2	105.8	89 sec (+ve)
8	MK	29	M	-	104	76	86	126
9	SS	2	F	-	84.3	78.0	125	122
10	PSO	22	F	-	94.8	102.3	98.4	128
11	CS	46	M-	92.6	74.3	110	129	
12	SI	37	M	Teratoma	104.6	96.2	82	124
13	PS	73	F	-	102.8	96.4	102	122
14	PPS	62	M	ALL	108.8	72.6	96.2	128
15	SKK	04	F	ALL	102.6	91.8	118	127
16	ASB	36	F	-	71.9	57.3	100	130
17	VS	51	F	-	82.3	75.4	102.4	124
18	SD	23	M	-	103.2	82.5	106.3	102 sec (+ve)
19	PKS	24	F	-	89.6	76.4	110.5	122
20	SS	21	M	-	102.5	87.4	102.8	138
21	SBC	30	M	-	78.4	92.8	110.0	92 sec (+ve)
22	GK	31	M	-	109.5	78.4	98.8	124

Two of these patients had APC-resistance and one each had Protein S and AT deficiency. The patient with AT deficiency, first developed acute MI and subsequently developed DVT and pulmonary embolism, for which he was successfully treated. This patient had three siblings. Family screening for AT deficiency revealed another male sibling (30 years) to be border-line deficient. He was however, asymptomatic. Neither his parents, nor two other female siblings and daughter were AT deficient. Another 20 year old male patient presenting with acute myocardial infarction, developed DVT three months following his discharge from hospital. He had APC-Resistance. All the 4 patients of stroke in young had CVT. Out of these four patients, one patient had APC-R and another had AT deficiency.

In all, out of 42 patients studied, 10 had APC-resistance (23.8%), 4 had AT deficiency (9.5%), 3 had Protein C deficiency (7.2%) and only 2 had Protein S deficiency (4.8%) (Table 4). Protein S deficiency was confirmed on repeat samples. After female patients with recurrent foetal loss were excluded, APC-resistance was found to be more common in females (4 / 12, 33%) than in males (6 / 24, 20.8%). Majority of deficiencies noted were in patients in the 3rd [n=9] and 2nd decade [n=4] of life. A total of 19 patients (45.2%) were found to have coagulation factor deficiency. No patient showed combination of two deficiencies in this study.

Discussion

Thromboembolic disorders may be secondary to Diabetes Mellitus, Vasculitis, PNH, estrogen therapy, DIC, antiphospholipid antibody syndrome, etc [5-7]. Most patients, have a single defect, but there may be a combination of defects [8,9]. The commonest cause of

Table 3**The thrombo-embolic conditions (N=20)**

S. No.	Patient	Age (yrs)	Sex	Clinical conditions	Protein C %	Protein S %	AT%	APC-R (sec)	Developed assoc conditions
1	MS	26	F	Recurrent foetal loss	87.4	96.2	104	92 sec (+ve)	Hemi paresis
2	SKS	21	F	-do-	92.4	82.8	118.4	124	-
3	DB	18	F	-do-	49.6	79.5	98.4	122	DVT leg
4	MT	28	F	-do-	78.3	84.5	93.5	129	-
5	SL	25	F	-do-	83.6	72.6	88.5	130	-
6	SS	31	F	-do-	94.5	85.6	114.5	125	-
7	RK	33	M	Stroke in young CVT)	102.8	69.9	64.0	127	-
8	PC	24	F	-do-	96.4	80	84.6	122	-
9	VS	28	M	-do-	104.5	79.6	89.4	95 sec (+ve)	DVT leg
10	SK	31	M	-do-	110.2	82.6	89.0	124	-
11	SL	20	M	Vaso occlusive coronary artery disease (CAD)	78.8	100.2	100	92 sec (+ve)	DVT
12	RS	24	M	-do-	86.7	89.7	56	126	Pulmonary Embolism with DVT
13	GS	19	F	-do-	71.6	47.3	108	124	-
14	SK	23	F	-do-	108.6	93.9	108	104 sec (+ve)	-
15	HR	52	M	Portal vein	82.6	105.2	101	124	-
16	RDS	10½	M	Hepatic vein	78.6	94.8	110	128	-
17	PCN	20	M	Nephrotic syndrome (NS)	130	95.8	144	124	DVT
18	RA	28	M	-do-	102.4	90.8	72.4	129	Recurrent DCT
19	SL	30	M	Pr. Pulmonary hypertension	106.4	82.4	98.2	124	-
20	AR	24	M	Pulmonary embolism	56.2	96.4	106.6	125	DVT

inherited thrombo-embolic disorder is APC-R, first discovered in 1993 by Dahlback [10] and inherited as an autosomal dominant trait. The basis for APC-R was identified as a mutant Factor V molecule (Factor V Leiden) in 1994 [11,12] and affects upto 5% of the Western Europe population [13]. In this study, out of the 42 cases, APC-R was seen in 10 cases (23.8%). It is the commonest cause of inherited thrombotic disorders [8]. Majority of these presented with a DVT (n=6), while others had vaso-occlusive coronary artery disease (n=2), recurrent foetal loss (n=1) and stroke in young (n=1). Venous thrombosis is the most common clinical symptom of APC-R and accounts for either cases of myocardial infarction, stroke or DVT in healthy young individuals [14]. APC-R accounts for 20% foetal loss [15]. Cerebral venous thrombosis in young can often be due to APC-R [16]. A recent study estimated that hazard ratios for venous thromboembolism in factor V Leiden heterozygotes and homozygotes compared with non carriers in adult Danish population was 3 and 18 respectively [17]. Acquired resistance to activated protein C has also been reported in patients with breast cancer [18]. Patients with recurrent thrombotic episodes require long term anticoagulant therapy. Asymptomatic patients with APC-R should not be treated, but female patients with this disorder should be informed about additional thrombotic risk associated with intake of oral contraceptives [8,9]. However, in women having factor

Table 4**Spectrum of coagulation inhibitors (N=42)**

Clinical diagnosis	Protein C deficiency	Protein S deficiency	AT-deficiency	APCR
DVT (n=22)	1 (4.5%)	1 (4.5%)	1 (4.5%)	6 (27.3%)
Other thromboembolic Condition (n=20)	2 (10%)	1 (5%)	3 (15%)	4 (20%)
Total	3 (7.1%)	2 (4.8%)	4 (9.5%)	10 (23.8%)

V Leiden associated with other risk factors for thrombophilia, prophylaxis is strongly indicated [19]. Hormone replacement therapy (HRT) increases the risk of venous thrombosis 15 fold in women with factor V Leiden and so it should be avoided [20]. Lately, highly sensitive tests to evaluate APC-R determined by thromboplastin based tests are under evaluation [21].

Anti-thrombin deficiency was detected in 4 out of the 42 cases studied which correlates well with other studies [8]. One presented with DVT while there was one each of stroke in the young, NS and vaso-occlusive coronary artery disease. AT deficiency (formerly known as AT-III) was discovered in 1965 by Egeberg [22]. The prevalence of AT deficiency in western population is 1 in 600 [23] and is inherited as an autosomal dominant trait [24]. The abnormal AT activity may be quantitative or qualitative [8]. The incidence of venous thromboembolism in AT deficient individuals is 20 times

higher [25]. AT deficiency can also be acquired in conditions such as DIC, liver disease and nephritic syndrome [26,27]. Long term management of these cases is by anticoagulant therapy. Patients with embolism may require fibrinolytic therapy. Asymptomatic patients may not need any treatment.

Protein C is a Vitamin-K dependent plasma protein. It can be activated by the thrombin-thrombomodulin system and the activated Protein C in turn inactivates coagulant factors, Va and VIIIa. This inhibits the coagulation cascade [28]. Protein C deficiency is an autosomal dominant disorder first described in 1981 by Griffin and co-workers [29]. Protein C deficiency may remain asymptomatic or may present as venous thrombo-embolism [30]. Treatment like in other such prothrombotic states requires the use of long term oral anticoagulants. Asymptomatic cases need not be treated. In our study 9% cases were due to Protein C deficiency which correlates well with other studies [8,10].

Protein S is also a Vitamin K dependent plasma protein and mediates the actions of activated Protein C, in that it inhibits factor Xa activity. It is also inherited as an autosomal dominant trait [31]. Protein C deficiency, is often associated with APC-R [32]. Protein S deficiency may present with a normal coagulation profile or with acute thrombo-embolic phenomenon. We had only two cases of Protein S deficiency (4.8%), one with vaso-occlusive coronary artery disease, while the other presented with DVT which correlates well with other studies [5,8,32].

To summarise, 42 cases of thrombophilia were evaluated. In literature, the cases of Protein C and Protein S deficiency slightly outnumber patients with AT deficiency, while the reverse was observed in our study, as the sample population evaluated is not large [8,13].

Family screening of a case of AT deficiency revealed a borderline AT deficient asymptomatic male sibling. The parents, two daughters and other siblings in this family were found normal on screening. Family screening if done can detect potential cases at risk, which can be educated and managed accordingly.

Conflicts of Interest

None identified

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Quiz

Radiological Quiz

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A newborn, full term normal delivery to non-consanguineous parents, mother 29 years old, 3rd gravida with no history of teratogenic exposure presented with shortening of right lower limb was referred for radiograph of lower extremities (Fig 1). The thigh segment of the right limb was short and held in flexion, abduction and external rotation at the hip.

What is your diagnosis?

Answer to the quiz - page 183

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