

## Homocysteine, Soluble Fibrin and D-Dimer Levels in Cerebrovascular Accidents



### Medical Science

**KEYWORDS :** Cardiovascular accidents (CVA), Homocysteine (Hcy), Soluble fibrin (SF), D – dimer, Disseminated Intravascular Coagulation (DIC), Deep Vein Thrombosis (DVT), Cardiovascular Accidents (CVA) and Thrombosis

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### ABSTRACT

*Increased homocysteine, soluble fibrin and D – dimer have been considered useful markers for the diagnosis of thrombosis. The present study was undertaken to evaluate serum homocysteine (Hcy), soluble fibrin (SF) and*

*Plasma D- dimer levels in patients with cardiovascular accidents. One hundred and three patients were enrolled for the study and thirty seven age and sex matched individuals free from cardiovascular diseases were taken as control group. Serum homocysteine (Hcy) levels were estimated by ELISA method using Axis homocysteine ELISA kit Manufactured by Rambaxy Diagnostic Ltd. India. Plasma concentration of soluble fibrin (SF) and D – dimer were measured by Cobas (Fully automated clinical chemistry analyser).*

*The findings suggest that serum homocysteine (Hcy) and concentrations of D – dimer and soluble fibrin (SF) were significantly high to diagnose patients associated with male preponderance which increase with advancing age.*

### INTRODUCTION

Homocysteine (Hcy) level is significantly higher in patients with stroke (cerebral infraction) than healthy controls. Hyperhomocystinemia occurs in patients of cerebrovascular accidents. Protein C activation and an increased oxidizability of LDL have been described as a few possible mechanisms by which homocysteine (Hcy) causes arteriosclerosis and thrombosis<sup>(1)</sup>. Fibrin – related markers such as D – dimer, fibrin and fibrinogen degradation products, soluble fibrin (SF) were considered useful for the diagnosis of thrombosis.

These markers are reported to be elevated in deep vein thrombosis (DVT) or pulmonary embolism (PE)<sup>(2-4)</sup> disseminated intravascular coagulation (DIC)<sup>(5-7)</sup> acute myocardial infarction (AMI)<sup>(8-9)</sup> and thrombocytopenic purpura<sup>(10)</sup>. D – dimer is widely used to diagnose thrombosis as deep vein thrombosis (DVT)<sup>(11-13)</sup>.

The present study has been undertaken to evaluate the serum total homocysteine (Hcy), plasma D-dimer and soluble fibrin (SF) in the diagnosis of thrombosis such as deep vein thrombosis, disseminated intravascular coagulation (DIC), cerebral thrombosis and acute myocardial infarction (AMI) respectively.

### MATERIALS AND METHODS

One hundred and three patients presenting with cerebrovascular accidents consisting of both hemorrhagic and infraction over a period of one year were enrolled in the study. Their detailed medical history was maintained regularly. All patients were subjected to high grade level of investigations including neurological examinations, brain CT scan and all routine blood examinations liver function tests, kidney function tests, urine examination, chest X – rays, ECG and EEG etc. Beside these other related examinations were performed as when required. The evaluation of previous cerebrovascular events and events that occurred during the study were performed according to the medical and health norms. Thirty seven age sex matched subjects who had no history or signs of cerebrovascular diseases were recruited to form the control group.

**Table 1: Sex wise distribution and Mean Age SD in cases and controls**

Study Group	Gender	Mean Age	SD	P
Cases (n=103)	Male (n=68)	62.19	13.12	0.113
	Female (n=35)			
Control (n=37)	Male (n=24)	57.85	9.65	
	Female (n=13)			

P > 0.05 (Insignificant)

**Table 2: Homocysteine, D-Dimer and Soluble Fibrin levels in cases and controls**

Subject	D-Dimer	Soluble Fibrin	Homocysteine	P
Normal	2.45 gmml <sup>-1</sup>	2.45 gmml <sup>-1</sup>	3.34 mmol/L	< 0.01
Cases	3.66 gmml <sup>-1</sup>	3.97 gmml <sup>-1</sup>	6.39 mmol/L	< 0.01
DIC	13.2 gmml <sup>-1</sup> (8.09 – 23.4 mgml <sup>-1</sup> )	17.73 gmml <sup>-1</sup> (9.30 – 32.96 mgml <sup>-1</sup> )	12.8 mmol/L (15.18 – 38.85 mmol/L)	< 0.01
DVT	10.5 gmml <sup>-1</sup> (4.41 – 18.4 mgml <sup>-1</sup> )	18.9 gmml <sup>-1</sup> (7.91 – 37.26 mgml <sup>-1</sup> )	13.3 mmol/L (7.9 – 45.5 mmol/L)	< 0.01
CVA	7.59 gmml <sup>-1</sup> (4.85 – 17.11 mgml <sup>-1</sup> )	9.43 gmml <sup>-1</sup> (7.26 – 16.44 mgml <sup>-1</sup> )	8.50 mmol/L (4.60 – 32.85 mmol/L)	< 0.01

\* Data are mean ± SD. CVA: Cerebral vascular accident because of thrombosis; DIC: Disseminated intravascular coagulation; DVT: Deep-vein thrombosis.

**Table 3: Underlying diseases of subjects**

Diseases	Age* (years)	Sex (F:M)	Thrombosis (%)	DVT (%)	DIC (%)	CVA (%)	Others (%)
Cancer	63.9 ± 15.1	124:105	31 (13.0)	17 (7.0)	10 (4.0)	3 (1.0)	1 (0.4)
Orthopedic diseases	57.8 ± 17.6	102:33	35 (22.9)	33 (21.6)	0	1 (0.7)	1 (0.7)
Hematological diseases	40.0 ± 14.8	30:23	12 (19.7)	4 (6.6)	3 (4.9)	4 (6.6)	1 (1.6)
Benign tumor	45.6 ± 14.1	28:16	3 (6.0)	3 (6.0)	0	0	0

Auto immune diseases	50.3 ± 14.2	34:7	1 (2.2)	1 (2.2)	0	0	0
Liver diseases	49.3 ± 9.5	14:27	0	0	0	0	0
Infectious diseases	62.7 ± 15.3	15:15	13 (40.6)	1 (3.1)	12 (37.5)	0	0
Digestive diseases	49.8 ± 18.5	11:18	5 (16.1)	4 (12.9)	0	1 (3.2)	0
Hypertension	58.7 ± 13.7	16:12	5 (17.2)	1 (3.4)	0	2 (6.9)	2 (6.9)
Heart diseases	66.2 ± 13.5	7:13	1 (4.8)	0	0	0	1 (4.8)
Diabetes mellitus	60.5 ± 12.6	6:4	5 (35.7)	2 (14.2)	0	17.1	2 (14.2)
Aneurysm and varicosity	61.4 ± 14.6	5:5	7 (58.3)	1 (8.3)	6 (50.0)	0	0
Trauma / burn	47.9 ± 20.6	1:6	3 (37.5)	1 (12.5)	1 (12.5)	0	1 (12.5)
Other diseases	57.4 ± 18.8	1:0	0	0	0	0	0
No underlying disease	56.6 ± 13.6	21:14	23 (60.5)	19 (50.0)	0	2 (5.3)	2 (5.3)

\* Data are mean ± SD. CVA: Cerebral vascular accident because of thrombosis; DIC: Disseminated intravascular coagulation; DVT: Deep-vein thrombosis.

**RESULTS**

Cerebrovascular accident patients and controls were well matched for age and gender (Table1). Cerebrovascular accident seems to have a male preponderance over female, having a ratio of 68:35. The mean homocysteine level is also higher in males than in females showing 6.70 ± 45 mmol/L and 5.70 ± 39 mmol/L respectively as seen in Table2. The maximum numbers of cases are in the age group 62 – 72 years with homocysteine levels of 7.2 ± 4.3 mmol/L. The highest Hcy level was observed in the age group 74 years and above. This implies that the level of homocysteine increases as age advances. When the levels of total Hcy are compared between the cases and controls, the study group has 6.32 ± 4.31 mmol/L whereas in the control, it is 3.51± 1.34 mmol/L which is statistically significant (P < 0.01).

Although the sample number was not ideal in this study, DVT was frequently associated with cancer and orthopedic diseases while DIC was frequently associated with cancer, infectious diseases and aneurysm. Such frequencies were similar to those reported in previous studies. With regard to the underlying diseases frequently associated with thrombosis (e.g. DVT and DIC), the risk for thrombosis should be evaluated by a simple test. In the present study, we demonstrated that the concentrations of both SF and D-dimer were significantly high in patients with thromboses such as DIC, DVT, CVA and AMI. Thus, high concentrations of SF and D-dimer could be considered as markers of thrombosis because both parameters were also reported to be elevated in DVT, DIC and hyperlipidemia. It should be noted, however, that there are no prospective studies that evaluated the SF assay including the cutoff value for the diagnosis of thrombosis. Plasma concentrations of SF and D-dimer were significantly high in patients with all types of thrombosis although the levels were also high in some patients who were found not to have any thrombosis. In the latter group, it should be noted that thrombosis cannot be diagnosed without complete or almost complete obstruction of the blood vessel and these patients might have thrombi that did not completely obstruct the vessel. There are many assays for D-dimer and the cutoff value depends on the assay.

A high false positive rate for the D-dimer can potentially result in an increase in pulmonary vascular imaging, increased length of stay in overcrowded emergency departments, and increased false-positive diagnosis of DVT or PE. Therefore, the cutoff values of SF and D-dimer for thrombosis should exceed the values above.

More than 50% of patients who had > 3.0 µg mL<sup>-1</sup> of D-dimer and > 6.0 µg mL<sup>-1</sup> of SF had some thrombosis, suggesting that these patients need anticoagulant therapy such as aspirin for atherosclerotic thrombosis or warfarin for venous thrombosis. It is considered that those patients with a high value of SF or D-dimer have hypercoagulable state. D-dimer is useful for the diagnosis of DVT but the cutoff values of D-dimer should be mentioned in each measurement kit. However, NPV was < 100% for low levels of D-dimer and SF, and PPV was < 85% for D-dimer and SF levels of 16 µg mL<sup>-1</sup>. These findings suggest that the diagnosis of SF and D-dimer was not perfect. That is, few patients with thrombosis had low SF or Ddimer values, and few patients who were not diagnosed with thrombosis had high values of D-dimer and SF.

With regard to the changes in D-dimer and SF, plasma levels of D-dimer were further increased after onset of DVT and were still high 7 days after the onset of DVT, while plasma SF levels diminished relatively soon after the onset. The SF reflects the early phase of DVT/PE while D-dimer reflects secondary fibrinolysis after clot formation. Therefore, measurements of both D-dimer and SF may be recommended. On the other hand, plasma concentrations of D-dimer correlated well with plasma SF concentrations, suggesting that secondary fibrinolysis occurs immediately after clot formation in almost all patients with DVT.

Our findings suggest that high concentrations of plasma fibrin-related markers, especially SF, also known as markers for hypercoagulable state, reflect high risk for thrombosis.

The frequency of thrombotic diseases was high in patients with solid cancer, orthopedic diseases, hematological diseases, infectious diseases, diabetes mellitus and aneurysm. DVT was frequently noted in patients with orthopedic diseases, digestive diseases and solid cancer, while DIC was frequently associated with infectious diseases and aneurysm (Table 3).

In healthy subjects, plasma concentrations of D-dimer and SF were not distributed normally, with maximum values 3.66 and 3.97 µg mL<sup>-1</sup>, minimum values 0 and 0 µg mL<sup>-1</sup>, and median values 0.66 and 0 µg mL<sup>-1</sup>, respectively. In healthy subjects, the 95% confidence intervals (95% CI) of D-dimer and SF were 2.45 and 2.45 µg mL<sup>-1</sup>, respectively. There were no significant differences in D-dimer and SF levels among various underlying diseases.

Plasma D-dimer concentrations (median; 25–75% percentile) were significantly higher in patients with DIC (13.2 µg mL<sup>-1</sup>; 8.09–23.4 µg mL<sup>-1</sup>), DVT (10.5 µg mL<sup>-1</sup>; 4.41–18.4 µg mL<sup>-1</sup>), OPE (8.76 µg mL<sup>-1</sup>; 3.04–13.2 µg mL<sup>-1</sup>), LT (8.02 µg mL<sup>-1</sup>; 2.82–13.11 µg mL<sup>-1</sup>) and CVA (7.59 µg mL<sup>-1</sup>; 4.85–17.11 µg mL<sup>-1</sup>) than in those without thrombosis (0.94 µg mL<sup>-1</sup>; 0.45–2.40 µg mL<sup>-1</sup>) (P < 0.01, each). Plasma SF concentrations were significantly higher in patients with DIC (17.3 µg mL<sup>-1</sup>; 9.30–32.96 µg mL<sup>-1</sup>), DVT (18.9 µg mL<sup>-1</sup>; 7.91–37.26 µg mL<sup>-1</sup>), OPE (13.32 µg mL<sup>-1</sup>; 8.17–25.84 µg mL<sup>-1</sup>), LT (15.94 µg minormL<sup>-1</sup>; 7.54–25.28 µg mL<sup>-1</sup>) and CVA (9.43 µg mL<sup>-1</sup>; 7.26–16.47 µg mL<sup>-1</sup>) than in those without thrombosis (2.32 µg mL<sup>-1</sup>; 0.69–4.92 µg mL<sup>-1</sup>) (P < 0.01, each). On the other hand, plasma con-

centrations of both D-dimer and SF were significantly higher in patients without thrombosis than in healthy subjects.

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