

Fibrinogen Levels In Ischemic Stroke

Dr. B.Haritha M. D¹, Dr. B.Sangeetha M. D², Dr. Matta Sreevani M. D³

*1*Assistant Professor, Department of Biochemistry, Siddhartha Medical College, NTRUHS, Vijayawada, India..

2 Assistant Professor, Department of Biochemistry, RIMS, NTRUHS, Srikakulam,, India..

3 Assistant Professor, Department of Biochemistry, Siddhartha Medical College, NTRUHS, Vijayawada, India

Abstract: Stroke is one of the leading causes of death and disability throughout the World. Several studies have clearly indicated that stroke does not occur suddenly, and there are many factors which precede stroke by several years. Hypertension is considered the main risk factor for cerebral thrombosis as well as cerebral hemorrhage. In most cases stroke is merely an incident in the slowly progressive course of a generalized vascular disease. Several prospective trials revealed that fibrinogen has a strong predictive power in ischemic stroke and numerous pathways have been identified through which fibrinogen can promote atherothrombosis. Some of the prospective studies suggest that there may be synergy between high plasma fibrinogen levels and hypertension in the onset of ischemic heart disease and stroke. The present study aims to estimate plasma fibrinogen levels in ischemic stroke patients and comparing with other risk factors. Present study enrolled 80 subjects including 50 patients of ischemic stroke and 30 healthy control subjects who are age matched and sex matched with patients. Patients clinically diagnosed to have ischemic stroke were selected for the study. Hemorrhagic stroke, traumatic and space occupying lesions of Cerebrovascular disease, Patients with previous MI or present Myocardial Ischemia, patients with recent infections, liver disease and renal failures are excluded. plasma fibrinogen is estimated by Fibroquant kit method. The study pursues correlations of significance between fibrinogen and various biochemical parameters including hypertension, age, smoking. The study showed plasma fibrinogen levels were significantly increased in ischemic stroke patients when compared to controls. The results were analysed by using chi-square test. p-value calculated and compared among both groups. p-value < 0.01 which is highly significant. 62.9% of patients with hypertension are found to have high fibrinogen levels. Thus hypertension and hyperfibrinogenemia in combination act as risk factors and contribute to the pathogenesis of ischemic stroke. Fibrinogen levels are found to be equally increased in both men and women showing no statistically significant gender difference. Fibrinogen is significantly raised 15 days after stroke which indicates that it is an acute phase reactant and has a role in the progression of atherosclerotic process in ischemic stroke patients. A small group of stroke patients (10%) have no conventional risk factors except hyperfibrinogenemia suggesting fibrinogen as an independent risk factor in ischemic stroke.

Keywords: Atherothrombosis, Cerebral hemorrhage, Cerebral thrombosis, Cerebrovascular disease, Fibrinogen, Hyperlipidemias, Hypertension, Ischemic heart disease, Stroke.

I. Introduction

The term "Stroke" is damage to part of the brain when its blood supply is suddenly reduced or stopped. WHO defined stroke as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting for more than 24 hours or leading to death, with no apparent cause other than vascular origin"¹. A Stroke may also be called a cerebrovascular accident, or CVA. Stroke is the most common clinical presentation in hospital admission out of which 85 – 90% are ischemic and 10-15% are haemorrhagic². Nearly 25% of patients having stroke die in a year and 15 – 30% are disabled. Incidence of stroke increases with age; doubling every 5 – 10 years beyond the age of 55 years³. Asians have high incidence of stroke than whites. Based on the retrospective analysis of subjects admitted in Urban hospitals in India, it was found that stroke constitutes nearly 2% of all hospital cases and 20% of neurological admission⁴.

Ischemic stroke is a major component of cerebrovascular accidents in our setting. Several epidemiological studies have been carried out in the literature regarding ischemic strokes. Conventional risk factors were widely studied. They are Hypertension, Diabetes Mellitus, Hyperlipidemias, Smoking, Overweight, family history, Endocarditis, sleep apnea, sickle cell anemia, cocaine use. Research has identified metabolic syndrome as doubling the risk of stroke. Also known as syndrome X⁵.

But recently fibrinogen is emerging as an "Independent and Major risk factor for thrombo atherosclerotic diseases and peripheral vascular diseases"^{6,7}. Many studies are available regarding fibrinogen levels as a risk factor in cardiovascular diseases. The major studies included are: North Wick Park Heart Study; PROCAM study ; GRIP's study; Gothenberg study and Framingham study. Fibrinogen levels were studied in

detail in both cardiovascular disease and stroke patients. Still fewer studies are available regarding role of fibrinogen in pathogenesis and as acute phase reactant in stroke patients.

In Indian scenario studies regarding fibrinogen in stroke are minimal. RK Jindal et al, New Delhi had studied levels of fibrinogen in haemorrhagic and ischemic stroke and found that fibrinogen levels were significantly raised in both the conditions⁸.

Mukesh Kumar Sharma et al, Bikaner had studied fibrinogen levels only in ischemic strokes and found it to be one of the major risk factors⁹.

A.R. Kumar et al (secunderabad) studied Fibrinogen levels in ischemic and haemorrhagic stroke and found significant elevation in patients with ischemic stroke.

Pai Mahesh et al (Banglore) studied Plasma Fibrinogen as a risk factor for ischemic stroke¹⁰.

Fibrinogen levels increases with age and also in combination with other major risk factors like smoking, hypercholesterolemia and hypertension. Interestingly it is also noted that higher levels of fibrinogen were noted in active progressive lesions of carotid arteries. Even in TIA patients the blood viscosity, blood rheology and hematocrit and fibrinogen levels are increased. This indicates fibrinogen is having a role in the primary pathogenesis of atheroma development. Dynamics of high molecular weight plasma proteins like fibrinogen in relation to vessel wall was well studied¹¹.

Sadoshima and Tanaka found fibrinogen, LDL deposited at cerebral artery bifurcation, and they observed that the deposition of fibrinogen in the intima was a very early finding and therefore might even precede the deposition of LDL cholesterol. Thus fibrinogen play an important part in early evaluation of stroke. Plasma fibrinogen levels persistently high even one year after stroke. Hence it will guide further progression of atherosclerosis in these patients and acts as a prognostic parameter¹².

All these studies have inspired us to undertake plasma fibrinogen estimation in ischemic strokes and comparing with other risk factors.

II. Aim Of Study

The present study aims to estimate plasma fibrinogen by Modified Cullen and Vanslyke method using Biuret Quantitation for standardization¹³ and Fibroquant kit method for estimation¹⁴. In addition other parameters estimated are fasting blood glucose, serum cholesterol, serum triglycerides; serum HDL, serum LDL ; serum VLDL; serum creatinine. The study pursues correlations of significance between fibrinogen and various biochemical parameters including hypertension, age, smoking.

III. Materials and Methods

Informed consent was taken from the patients in their own language before collecting data. This study was approved by Ethics Committee of Siddhartha Medical College, Vijayawada. Present study enrolled 80 subjects including 50 patients (with mean age 55.54 years) of ischemic stroke admitted in Medical Wards and casualty of Govt. General Hospital, Vijayawada. Out of 50 cases 33 are men and 17 are women. The study also included 30 healthy control subjects who are age matched (mean age 56.2 years) & sex matched with patients. Out of 30, 20 are men and 10 are women.

A detailed history taken considering various risk factors for stroke like hypertension, smoking, DM, alcoholism and previous stroke from study group.

3.1. Inclusion Criteria:

3.1.1. Age : 50 - 60 Yrs

3.1.2. Patients clinically diagnosed to have ischemic stroke were selected for the study.

3.2. Exclusion Criteria:

3.2.1. Hemorrhagic stroke, traumatic and space occupying lesions of Cerebrovascular disease are excluded from study after taking CT Scan.

3.2.2. Patients with previous MI or present Myocardial Ischemia and patients with recent infections, liver disease and renal failures are excluded.

3.3. Collection of Samples:

3.3.1. Blood sample for plasma fibrinogen was taken within 24-72 hours of admission and repeated after 15 days and fibrinogen is estimated with Fibroquant kit. Sample collected without undue venous stasis and without frothing into a new plastic syringe fitted with a short needle of 20 SWG. Nine parts of freshly collected blood (1.8ml) was mixed with one part of Sodium Citrate (0.2ml). Plasma was separated and immediately tested

Principle: The addition of thrombin coagulates fresh citrated plasma. The coagulation time is proportional to the fibrinogen concentration. This allows the estimation of plasma fibrinogen by functional clotting assay.

Normal Range: Fibrinogen is 200 - 400 mg/dl .

3.3.2. Fasting blood sample collected for lipid profile and blood sugar on the next day of admission. 5ml of venous blood collected in plain bottle and serum separated. Fasting blood sugars [FBS] and Post prandial blood sugars [PPBS] were estimated by GOP-POD Method By Spectrophotometer.

$$\text{Glucose Conc. in mg/dl} = \frac{\text{Abs of T}}{\text{Abs of S}} \times 100$$

Normal Range: Fasting :70 – 110 mg/dl
Post prandial:< 140 mg/dl

Cholesterol and HDL Cholesterol are estimated by CHOD/POD – PHOSPHOTUNGSTATE METHOD (Monozyme Kit)

$$\text{Total Cholesterol (in mg/dl)} = \frac{\text{Abs of Tc}}{\text{Abs of S}} \times 200$$

$$\text{HDL Cholesterol (in mg/dl)} = \frac{\text{Abs of T}_H}{\text{Abs of S}} \times 50$$

Normal Range:

Total Cholesterol : 130 – 250 mg/dl

HDL Cholesterol: 30 – 70 mg/dl – Male
35 –90 mg/dl – Female

Serum triglycerides are estimated by Glycerol –3-phosphate oxidase / N-ethyl-N-sulphopropyl-n-anisidine (GPO/ESPAS, 1973) method (Monozyme Kit)

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs.of T}}{\text{Abs. of S}} \times 200$$

Normal Range: Serum Triglycerides : 80—180 mg/dl

3.3.3. Blood Pressure recorded in Right Upper limb in supine position with Sphygmomanometer.

Normal Range: Blood Pressure : $\frac{120 \pm 20}{80 \pm 10}$ mm of Hg

Plasma fibrinogen, lipid profile, Blood Sugar are estimated in control group also.

All parameters are analysed immediately after collection of samples.

IV. Results

The present study enrolled total 80 individuals. 50 were cases of ischemic stroke and 30 were normal subjects as controls. The results were shown in tables and figures given below. The results were analysed by applying Chisquare test. P- value calculated and compared among two groups.

Table – I: Plasma Fibrinogen Levels In Cases & Controls

	Range mg/dl	Mean mg/dl	SD	SEM
Cases n=50	290-700	498.1	118.22	16.72
Controls n=30	200-350	310.3	34.37	6.28

t value : 10.512
p value < 0.01

In the present study, mean value of fibrinogen among cases (average) is 498.1 mg/dl ± 118.22 (Mean ± SD) with range of 290-700 mg/dl & that of controls is 310.3mg/dl ± 34.37 with range of 200-350 mg/dl. The increase in plasma fibrinogen among cases is significant with a P value of < 0.01

Table – II: Plasma Fibrinogen Levels In Male & Female Cases

	Range mg/dl	Mean mg/dl	SD
Male n=33	350-675	503.6	105.53
Female n=17	290-700	487.2	140.14

The mean value of plasma fibrinogen in males is 503.6± 105.53 & in females is 487.2 mg/dl ± 140.14. Though males show apparently elevated value, the difference is not statistically significant.

Table – III: Plasma Fibrinogen Levels In Ischemic Stroke Patients

Range mg/dl	No. of Males	33%	No. of Females	17%	Total	50%
200 – 299	-		1	6%	1	2%
300 – 399	9	27%	6	35%	15	30%
> 400	24	73%	10	59%	34	68%

Total patients with plasma fibrinogen level > 400 mg% are 34 out of 50 constituting 68%; males are 24 (73%) and females are 10 (59%)

Table –IV: Fibrinogen & Lipid Profile In Cases & Controls

	Mean Values		t Value	p Value
	Cases	Controls		
Plasma Fibrinogen mg/dl	498.1	310.33	10.512	< 0.01
LDL mg/dl	145.1	106.33	6.45	<0.05
Total Cholesterol mg/dl	215.9	184.73	5.24	<0.05
HDL mg/dl	41.4	59.24	8.36	<0.01

The table shows comparison of various parameters in cases and controls with statistical significance.

Table –V: Fibrinogen – As Acute Phase Reactant

Fibrinogen mg/dl	Total n=50 Mean mg/dl	Males n = 33 Mean mg/dl	Females n =17 Mean mg/dl
1 st Sample (F ₁) 1 st Day	397.2	400.0	391.8
2 nd Sample (F ₂) 15 th Day	598.9	607.3	582.7

In the study, the plasma fibrinogen levels are more in the second samples compared to first sample. plasma fibrinogen levels are more in the second samples compared to first sample.

Table –VI: Risk Factors In Ischemic Stroke

Risk Factor	Patients n =50	%	Males n =33	%	Females n = 17	%
Hypertension	35	70%	24	72%	11	64%
Smoking	28	56%	27	96.4%	1	100%
Hypercholesterolemia	39	78%	28	84%	11	64%
DM	5	10%	1	3%	4	23%
LDL	37	74%	28	84%	9	52%
No Risk Factor	5	10%	1	3%	4	23.5%

In the study, it is shown that hypercholesterolaemia constitute 78% , LDL 74%, HTN 70%, smoking 56% & DM 10%. So it has been inferred that hyperfibrinogenemia is associated positively with HTN, hypercholesterolaemia, LDL, smoking and DM as shown below

Table – VII: Hyperfibrinogenemia Association With Major Risk Factors

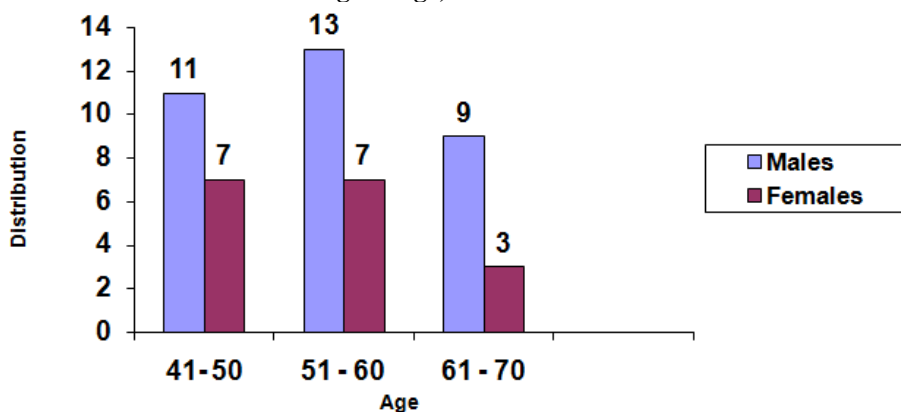
Risk factor	No. of Patients	Patients with increased fibrinogen	Percentage
Hypertension	35	22	62.9%
Smoking	28	24	85.7%
Hypercholesterolaemia	39	27	69.2%
LDL cholesterol	37	25	67.6%
Diabetes Mellitus	5	4	80%
NO R/F	5	4	80%

Table – VIII: Risk Of Hyperfibrinogenemia With Age & Gender

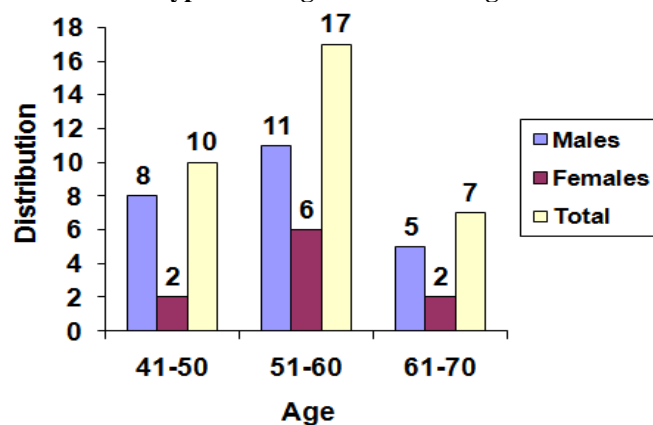
Age Group	Males with increased fibrinogen %	Females with increased fibrinogen %	Total with increased fibrinogen %
41-50	(11) 8 → 72%	(7) 2 → 28%	(18) 10 → 55%
51-60	(13) 11 → 84%	(7) 6 → 85%	(20) 17 → 85%
61-70	(9) 5 → 55%	(3) 2 → 66%	(12) 7 → 58%
Total	(33) 24 → 72%	(17)10 → 58%	(50) 34 → 68%

According to the present study, elevated levels of plasma fibrinogen is more in the age group of 41-60 years in males , in females in age group of 51-60 years.

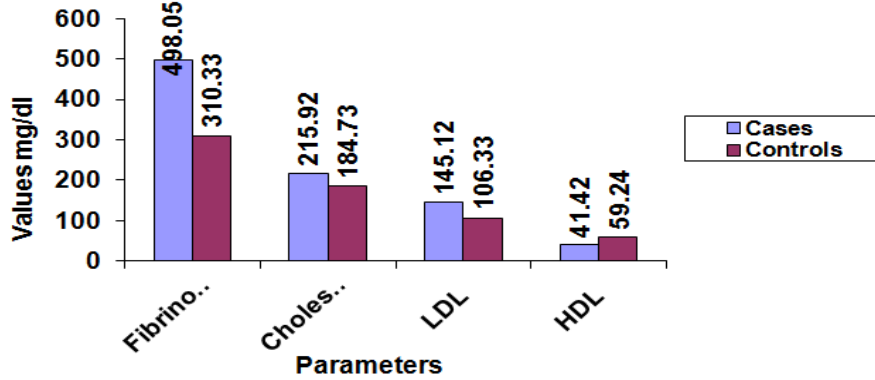
Bar Diagram –I
According To Age, Gender Distribution



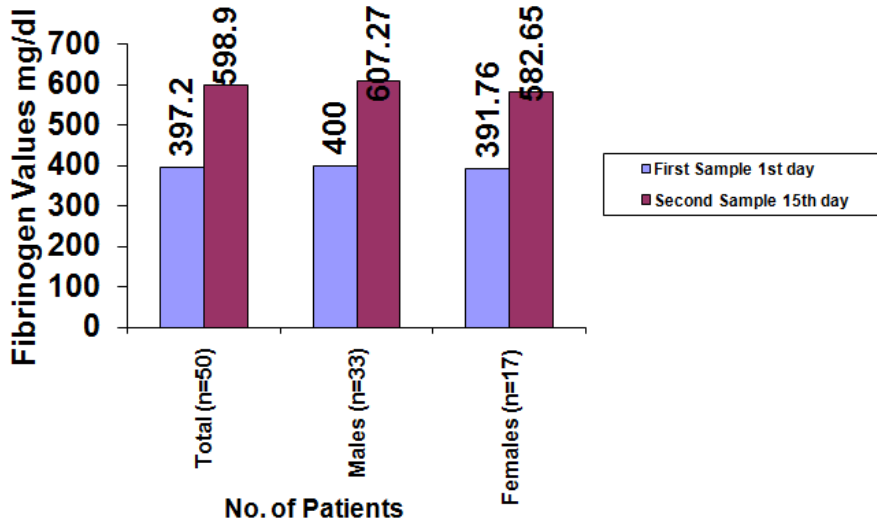
Bar Diagram –II
Relation Of Hyperfibrinogenemia With Age And Gender



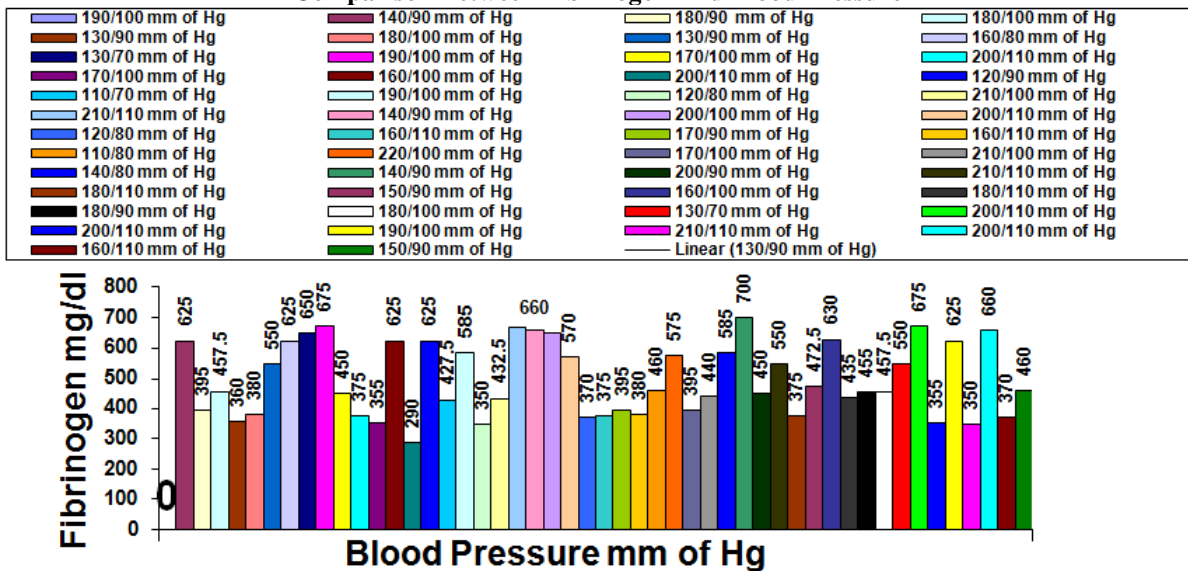
Bar Diagram –III
Comparison Between Fibrinogen And Lipid Profile In Cases And Controls

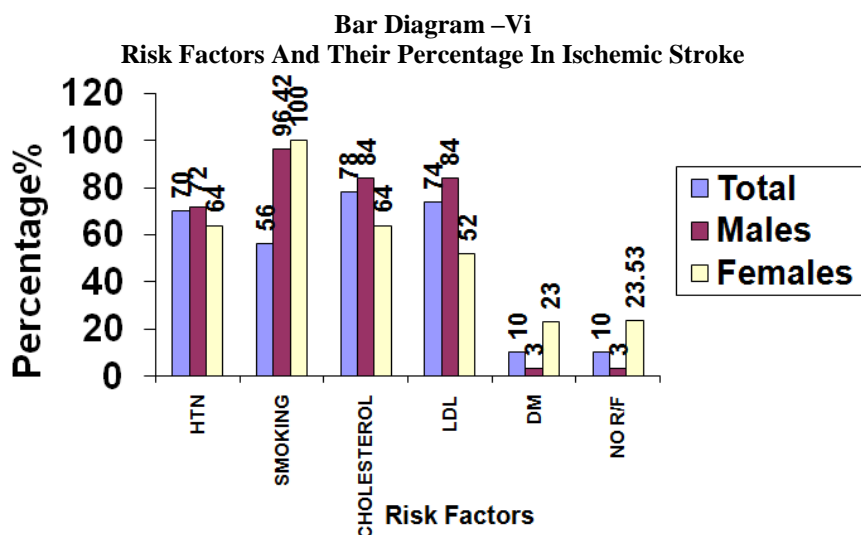


Bar Diagram –Iv
Fibrinogen As Acute Phase Reactant



Bar Diagram –V
Comparison Between Fibrinogen And Blood Pressure





V. Discussion

In present study 50 Ischemic stroke patients were studied for the plasma fibrinogen levels as a risk factor, as an acute phase reactant and its association with other risk factors. 30 age matched controls were taken for comparison. Present study included 33 male patients and 17 female patients. Age group varied from 41-70 patients, majority falling between 51-60 years. On observation of isolated risk factors, the following descending order was noted. Hypercholesterolaemia (78%), LDL (74%), Hypertension (70%); Hyperfibrinogenemia (68%), Smoking (56%) and Diabetes Mellitus (10%) Mean plasma fibrinogen levels (397.2 mg/dl – 1st sample) are elevated in the cases (498.05 mg/dl – average) compared to controls.

34 out of 50 patients studied shown a significant rise of fibrinogen levels constituting 68%; males comprised of 24 out of 33 i.e., 72.7% showed elevated fibrinogen levels. Females comprised of 10 out of 17 i.e., 58.8% showed elevated fibrinogen levels. A.R.Kumar et al in their study on plasma fibrinogen level in stroke showed elevated fibrinogen levels in 65% of their total patients; males are 80% & females 50%. These results are in concordance agreeing with the present study.

Present study shows that hyperfibrinogenemia is associated with risk factors like hypertension, hypercholesterolemia; LDL; smoking and diabetes mellitus. It is seen that hypertension is seen in 62.9%; hypercholesterolemia in 69.23%; LDL in 67.6%; smoking in 85.7% and diabetes in 80% of total cases. Thus these are positively associated with hyperfibrinogenemia. HDL is negatively associated. Almost similar results are shown in their study by A.R.Kumar et al, i.e., hypertension 68.1%; smoking 100% DM 75%; hypercholesterolemia 60% & high LDL in 68.75%.

Age wise comparison of fibrinogen has shown that 85% of patients are in the age group of 51-60 years has hyperfibrinogenemia (> 400 mg/dl) and 58% in the age group 61-70 years and 55% in the age group 41-50 years showing increase of fibrinogen with age; thus increasing the risk for stroke. Spada et al have shown that patients with ischemic stroke have an earlier and more evident age related increase in fibrinogen values than controls, showing fibrinogen is an important risk factor in elderly for stroke

In the present study elevation of mean fibrinogen in males (400 mg/dl) than in females [391.76 mg/dl 1st sample] is not significant. But Framingham study has shown fibrinogen mediated risk declined in women compared to men. Present study have not shown such gender variation. Fibrinogen levels were studied individually in males and females.

Lars Wilhelrogen has shown that blood pressure and fibrinogen when combined were significant risk factor. Present study agrees with the same (62.9%).

More recurrent stroke or CAD complications are noted in patients who have persistent elevation of plasma fibrinogen (Mario Dinapoli). Framingham study has shown association between hyperfibrinogenemia and smoking in males and risk doubles with the amount of smoking. The present study also inferred that smokers have higher fibrinogen amounting to 96.4% in males and 100% in females.

45 out of 50 patients i.e., 90% carry one or more of the major risk factors. But 5 out of 50 (10%) shown no specific conventional risk factors, among them 3 had hyperfibrinogenemia giving us the indication that fibrinogen may itself be considered as risk factor for ischemic stroke.

In the present study high cholesterol 69.2% and LDL 67.6% were positively associated with hyperfibrinogenemia which is also shown by Engstrom et al. Men with hypercholesterolemia with combination of fibrinogen or CRP etc., had an increased risk of ischemic stroke association of hyperfibrinogenemia with increasing age, smoking and BP is shown its UK TIA trial and Dutch TIA trial.

Nawab Qizilbash et al had shown that high total cholesterol is associated with hyperfibrinogenemia in ischemic stroke patients. Similar results are observed in present study.

Fibrinogen levels are reestimated after 15 days in all patients and was observed that the mean value of fibrinogen is significantly higher in 2nd sample. 598.9 mg/dl than 1st sample 397.2 mg/dl. This shows that fibrinogen level is increased after stroke as an acute phase reactant protein. Persistent elevation of plasma fibrinogen levels after stroke will have a prognostic index.

Pai Mahesh et al conducted in their study that elevated fibrinogen is a risk factor for ischemic stroke and conventional risk factor like HTN, smoking, DM influence plasma fibrinogen levels positively. In the present study apart from the above findings positive correlation is also noted with risk factors like hypercholesterolemia and high LDL and negative correlation with HDL.

In comparison to synergy of risk factors in the pathogenesis of atheroembolism the following observation are noted, fibrinogen and smoking synergy is 85.7%; fibrinogen and cholesterol synergy is 69.23%; fibrinogen and LDL synergy is 67.6% and fibrinogen and hypertension synergy is 62.9%.

Large scale euro stroke project: In its recent study showed that fibrinogen is powerful predictor of stroke including fatal and non fatal and hemorrhagic and ischemic stroke. It is shown that hypertension and high fibrinogen is most dangerous combination, present study had shown similar association in ischemic stroke.

VI. Conclusion

The plasma fibrinogen levels were significantly increased in ischemic stroke patients when compared to controls. There seems to be a strong association of rise of fibrinogen in smokers (85.7%). Hyperlipidemias are positively associated with rise in fibrinogen in ischemic stroke patients (69.2%). Especially LDL cholesterol & Total Cholesterol were raised in conjunction with fibrinogen. Significant negative relation was noted with HDL levels. 62.9% of patients with hypertension are found to have high of fibrinogen levels. Thus hypertension and hyperfibrinogenemia in combination act as risk factors and contribute to the pathogenesis of ischemic stroke. Fibrinogen levels are found to be equally increased in both men and women (72.7% and 58.8% respectively) showing no statistically significant gender difference. Hyperfibrinogenemia is observed in 85% of patients in the age group of 51-60 years. Fibrinogen is significantly raised 15 days after stroke which indicates that it is an acute phase reactant and has a role in the progression of atherosclerotic process in ischemic stroke patients. A small group of 3 stroke patients (10%) have no conventional risk factors except hyperfibrinogenemia suggesting fibrinogen as an independent risk factor in ischemic stroke.

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