Original Article

Internal Medicine

A study on the blood levels of homocysteine, fibrinogen and hsCRP in diabetic patients with ischaemic stroke from eastern India

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ABSTRACT

Introduction: Diabetes is an important risk factor for ischaemic stroke. Newer risk markers like C - reactive protein, fibrinogen and homocysteine levels are now being considered for better risk predication.

Aim: To study these new risk markers in diabetic patients with ischaemic stroke and to study any association of these markers with other blood parameters. This was thus a case control study.

Methods: Patients who were proved (by imaging) to be suffering from cerebral ischaemic events were chosen after proper screening and after their consent was taken. The diabetic subset was compared with the non-diabetic subset by doing blood tests which included blood glucose, lipids and newer markers. This was done 2 months after the index event to avoid false positive results.

Statistical analysis: We analyzed the data by using online software. Pearson's correlation coefficient was used for finding the correlation between the variables.

Results: 82 patients were included in the study, of which 42 were diabetic (ADA Criteria). The diabetic subset had significantly higher levels of total serum cholesterol (186 ± 50.3 vs. 167± 30.6; p=0.041) and LDL levels (112 ± 33.3 vs. 92± 15.2; p=0.0008). The triglyceride levels were also higher (165± 19.35 vs. 124.6± 9.22; p=0.0010). The HsCRP and fibrinogen levels were higher in diabetic patients with ischaemic stroke ($p<0.05$), while the homocysteine levels were higher in the non-diabetic subsets. The high hsCRP levels also correlated significantly with blood glucose (for FBS; r= 0.288; for PPBS, r=0.407) and blood pressure. There was also a positive correlation between hsCRP and the fibrinogen levels (r=0.307; $p<0.05$). The ROC curve analysis showed that LDL values which were >110 mg/dl had a high sensitivity in predicting high levels of plasma homocysteine. The logistic regression model showed that hsCRP had the strongest correlation with increasing age (OR=1.1).

Conclusion: This case control study has shown significantly higher hsCRP and fibrinogen levels in ischaemic stroke patients who had diabetes, as compared to the non-diabetic subsets. These newer parameters were also correlated with blood glucose and the lipid values. Thus, these can be used as surrogate markers in diabetic patients for the prediction of ischaemic stroke. However, a prospective study is needed to identify the risk factors and their predicative value better.

Key Words: hsCRP, homocysteine, fibrinogen, stroke, diabetes, blood glucose.

KEY MESSAGE

- A proinflammatory state in diabetes predisposes to vascular events like stroke. The estimation of these new risk factors can help in better risk prediction in diabetic patients.

INTRODUCTION

Diabetes is a significant risk factor for ischaemic stroke, particularly in the young [1,2]. Associated factors like hypertension and dyslipidaemia predispose to stroke in a synergistic way with hyperglycaemia [1]. A study from south India has shown that diabetes as a risk factor has an odds ratio of 4.55 for causing ischaemic stroke [2].

Now, some newer risk factors are also being considered to be important in the pathophysiology of the vascular events. Plasma homocysteine levels are considered to be a vascular risk factor which is independent of hyperglycaemia [3]. Homocysteine is implicated in both coronary and cerebrovascular events, particularly in association with hypertension [4]. The plasma levels of another marker, fibrinogen correlate with the parameters of thrombin activation [5]. The fibrinogen levels also correlative positively with hypertension and cholesterol levels [6]. Including the C - reactive protein (CRP) for the risk assessment of cardiovascular events in diabetes gives a better predicative value [7]. CRP is a marker of endothelial activation and inflammation [8,9].

Thus, the measurement of these novel risk factors can be a new method to predict the risk in diabetes.

This study was conducted to assess the effect of homocysteine, fibrinogen and hsCRP levels along with other risk factors for atherosclerosis, in diabetic patients with ischaemic stroke and to compare these values with the values in non-diabetic patients with ischaemic stroke.

MATERIALS AND METHODS

We selected 82 patients of ischaemic stroke (proved by CT scan and/or MRI brain), who were admitted to the Department of

Medicine over a period of one year, from 30th November, 2009 to 31st October, 2010. The patients were divided into two groups: diabetic and non-diabetic. Only the patients whose ischaemic stroke was proved by CT scan or MRI of the brain were included in this study. Patients with suspected infection, HIV and rheumatological disorders like SLE, those who were on steroids and those who had known clotting disorder, overt signs of vitamin deficiency or malnutrition or pregnancy were excluded from our study.

After a proper clinical examination and after checking the past treatment records, the patients were subjected to laboratory tests like complete blood count, estimation of fasting and two hour post prandial blood glucose levels (measured by the glucose oxidase method), HbA1C% (determined by electrophoresis), blood lipid levels i.e. cholesterol, LDL, HDL and triglycerides (LDL was estimated by the cholesterol oxidase method, triglycerides by the glycerol peroxidase method and HDL by the PEG precipitation method), routine urine examination and ECG. The blood glucose and lipid levels were measured within 12 hours of the event, or after 4 weeks. The homocysteine, fibrinogen and hsCRP levels were also measured in all the patients. The fibrinogen levels (Normal range: 200-400 mg/dl) were measured by using Beckman Coulter Synchron CX5 pro, USA. The HsCRP levels were measured by an immuno-turbidimetic method and the cut off value of hsCRP was chosen as 3 mg/L, as the values above this were found to confer an increased vascular risk. The homocysteine levels (normal values were taken here as < 15 micromole/L) were measured in the fasting state, as homocysteine was observed to stay relatively constant over time for individuals who were in a stable state of health without dietary changes and as a protein rich meal could induce increases in the homocysteine levels for the next several hours.

These measurements were done at least 2 months after the event to avoid falsely elevated values in the acute phase. Diabetes was diagnosed according to the American Diabetes Association's guideline, 2010 [10].

However, reactive hyperglycaemia was not considered. For this, any high blood glucose value (except Hb A1C) was rechecked at least 5 days after the event to confirm the diabetic status. Also, the past records of the diabetics were considered.

The data was arranged in a Microsoft Excel worksheet. The analysis was done by using software like MedCalc, Graphpad and epiInfo, which were downloadable from the internet. The Student's t test was used to find the significant difference among the continuous variables between the 2 groups. The categorical data was arranged in 2*2 contingency tables and these were analyzed by using Chi square tests.

P-values which were less than 0.05 were considered to be significant.

RESULTS

There was a total of 82 patients of ischaemic stroke in our study, with a sex ratio of 50 males: 32 females. Forty two patients were diagnosed to be diabetic according to American Diabetic Association (ADA) criteria as per the blood test results [11]. The average age of the study population was 62.3 ± 9.75 years. [Table/Fig 1] shows the comparison of the various parameters in between the two groups. It was seen that the diabetic subset had significantly higher levels of total serum cholesterol (186 ± 50.3 vs. 167± 30.6; p=0.041) and LDL levels (112 ± 33.3 vs. 92± 15.2; p=0.0008). The triglyceride levels were also higher (165± 19.35 vs. 124.6±

Parameter	Diabetic	Non-Diabetic Subset	P-Value
AGE (yrs)	62.5 ± 5.83	62.1 ± 12.7	0.85
CHOLESTEROL (mg/dl)	186 ± 50.3	167± 30.6	0.041
LDL (mg/dl)	112 ± 33.3	92± 15.2	0.0008
HDL (mg/dl)	43.4 ± 8.57	39.8± 8.87	0.0717
TRIGLYCERIDES (mg/dl)	165± 19.35	124.6± 9.22	0.0010
SBP	178 ± 18.2	174± 26.6	0.48
DBP	99.8 ± 9.39	101± 17	0.76
BMI (kg/m2)	24.4± 1.8	24.4± 3.31	0.88

[Table/Fig-1]: Table showing the general characters of the two groups in study. *(SBP=Systolic Blood Pressure; DBP= Diastolic Blood Pressure; LDL= Low Density Lipoprotein; HDL= High Density Lipoprotein)*

Parameter	Diabetic	Non-Diabetic Subset	P-Value
hsCRP	3.29± 2.28	1.55± 0.86	0.0003
Homocysteine	12.5 ± 3.25	21.1 ± 9.65	<0.0001
fibrinogen	448.78 ±51.72	389.9 ± 63.77	0.0001

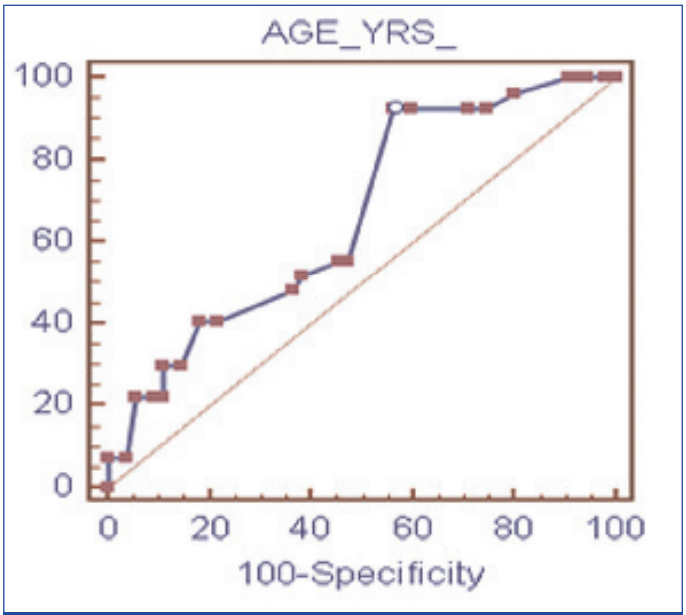
[Table/Fig-2]: Table showing the study parameters in the 2 groups.

Correlation	HSCRP	Fibrinogen	Homocysteine
Age	r=0.27; p=0.0128	r=0.215;p=0.051	R=0.13;p=0.24
SBP	r=0.21;p=0.0595	R=0.24; p=0.0242	R=0.14; p=0.23
DBP	r=0.104;p=0.35	R=0.278;p=0.0112	R=0.05;p=0.63
CHL	r=0.11; p=0.31	R=0.08; p=0.45	R=0.34;p=0.0019
LDL	R=0.12;p=0.412	R=0.05; p=0.9	R=0.45;p<0.0001
FBS	r=0.288;p=0.0105	R=0.22; p=0.0454	R=-0.25;p=0.12
PPBS	r=0.407; p=0.0002	R=0.33; p=0.0032	R=-0.3;p=0.254

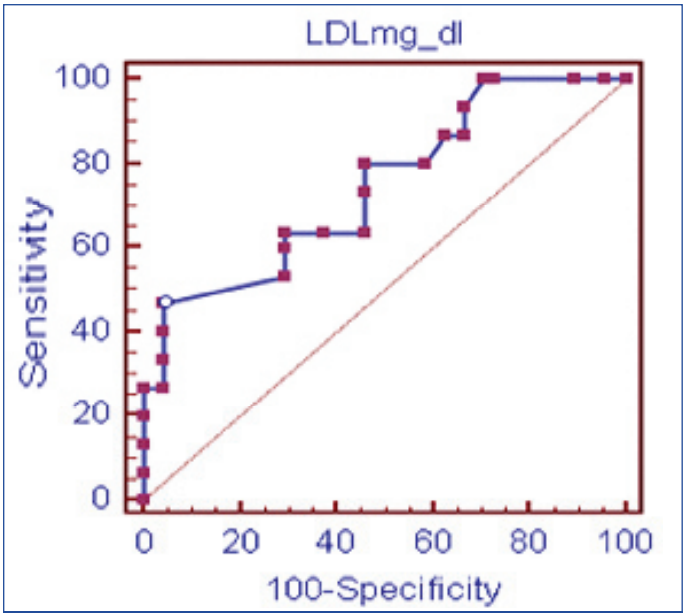
[Table/Fig-3]: Table showing the correlation of the study parameters with the other variables in the study population(r=coefficient of correlation).

Correlation	Fibrinogen	Homocysteine
hsCRP	R=0.307; p=0.005	R=-0.302; p=0.0058
Fibrinogen	----------	R= - 0.25; p=0.021
homocysteine	R= - 0.25; p=0.021	--------

[Table/Fig-4]: Table showing correlation between the study variables.



[Table/Fig-5]: ROC curve for age wit hsCRP levels.



[Table/Fig-6]: ROC curve for homocysteine with LDL levels.

9.22; p=0.0010). The systolic blood pressure was also higher in diabetics, but the difference were not significant (p=0.48).

The study parameters in the two groups are shown in [Table/Fig 2]. It was seen that in the diabetic subset, the hsCRP and the fibrinogen levels are significantly higher, while the homocysteine levels were significantly higher in the non-diabetic subset (12.5 ± 3.25 vs. 21.1 ± 9.65; p<0.0001). In [Table/Fig 3], the correlation of the hsCRP, fibrinogen and the homocysteine levels have been made with the other conventional risk factors among all the patients who were included in the study. The correlation study of hsCRP, fibrinogen and homocysteine showed that blood glucose had a strong correlation with these parameters. While for hsCRP and fibrinogen, the correlation was positive, for the homocysteine levels, this was not seen. This correlation was shown for both the FBS and the PPBS levels in the variables. The blood lipid levels correlated with the homocysteine values only (for total cholesterol, r=0.34, for LDL, r=0.45). The blood pressure, especially the systolic pressure (SBP) showed a positive correlation with the hsCRP and the fibrinogen levels (for hsCRP, r=0.21; for fibrinogen, r=0.24 for SBP). The ages of the patients were also correlated with the hsCRP(r=0.27) and the fibrinogen (r=0.215) levels. BMI (Body mass Index) did not show any significant association. These values resulted from the independent analysis of each variable. When the logistic regression model was used, the odds ratio for the high fibrinogen levels was 1.03 for high PPBS and it was 1.07 for the older age patients (p=0.0036). For the homocysteine levels, the odds ratio for LDL was 1.02 (p<0.0001). For the hsCRP levels, the odds ratio in case of age was the strongest (OR=1.102; p<0.0001), while for PPBS, it was 1.02. By using the ROC curve analysis (Figure 1), it was found that age which was >59 years had a sensitivity of 93% in predicting high CRP levels; however, the specificity was only 43% (p=0.008). A similar ROC curve analysis (Figure 2) also showed that LDL values which were >110 mg/dl had a specificity of 94% in predicting the high homocysteine levels (p<0.0001). The correlation data between the 3 study variables showed that (Table 4) there was a positive correlation between hsCRP and fibrinogen (r=0.307), while for homocysteine, the correlation was negative (r=-0.302; p=0.0058). This negative correlation of homocysteine was also seen with fibrinogen, although the correlation was less strong (r=0.25).

DISCUSSION

In our cross sectional study, we studied the different blood parameters in patients of ischaemic stroke and compared the results between the diabetic and the non-diabetic population. We found significantly higher levels of cholesterol, LDL and triglycerides in the diabetic population; also, the levels of hsCRP and fibrinogen were significantly higher. However, the serum homocysteine levels were significantly higher in the non-diabetic sub-group. These three vascular risk markers, hsCRP, fibrinogen and homocysteine showed a significant correlation with factors like age, pressure and blood sugar. Also, there was a positive correlation between the hsCRP and the fibrinogen levels.

Diabetes has been proved to be a great risk factor for stroke in all the age groups [1,2]. In a study from West Bengal, it was seen that hypertension with diabetes was significantly related to ischaemic stroke [11].

The novel risk factors hsCRP, fibrinogen and homocysteine are gaining importance as the predictors of vascular events beyond the blood glucose levels. Especially, the hsCRP levels have been shown to be the markers of atherosclerosis in different studies [8]. In our study, we found significantly high levels of hsCRP (3.29± 2.28 mg/L) in the diabetic subset. In different studies, it was shown that hsCRP was independently associated with stroke or other vascular events over a 7 year follow up [7]. The HsCRP levels in the presence of diabetes has an even greater significance [8,9].

Fibrinogen is also a marker of altered vascular physiology in diabetes [6]. A study from Italy showed that diabetic subsets had increased levels of fibrinogen and that it correlated with the HbA1c levels [6]. In our study, we did not find this correlation, probably due to the small number of subjects. The fibrinogen levels were also found to correlate with a higher risk of ischaemic stroke, which was independent of the blood glucose levels [12]. In the same study, hsCRP was found to be an important prognostic factor, not just a risk marker [12]. The CRP levels could identify those patients whose inflammation system responded most actively to stimuli [12].

In our study, we found lower levels of homocysteine in the diabetic subsets (12.5 ± 3.25 vs. 21.1 ± 9.65; p<0.0001). The correlation of diabetes and homocysteine has been debated. In some studies, the homocysteine levels in diabetic patients have been found to predict cardiovascular events strongly [4]. However, other studies have shown low homocysteine levels in the diabetic populations, especially in the young, type 1 diabetics [13]. The levels of homocysteine in diabetics are also influenced by their insulin concentrations, the therapy with insulin, and medications such as metformin and glitazones that can either raise or lower the homocysteine levels [14]. In animal experiments, the plasma homocysteine levels were reduced in streptozotocin-induced diabetic rats and they were increased with the institution of insulin treatment [15].

Plasma homocysteine is now known to be an important vascular risk factor [4]. In our study, we found a significant correlation of homocysteine with blood lipid levels (for cholesterol, r=0.34; for LDL r=0.45; p<0.05). This correlation, esp. with LDL was also shown in other studies [16].

HsCRP levels are directly related to insulin resistance. This was reflected in the significant positive correlation of CRP with fasting and post-prandial blood sugar levels in our study (for FBS; r= 0.288; for PPBS, r=0.407). This correlation of CRP with insulin resistance and other components of the metabolic syndrome were

also documented in other studies [7]. Another study from Germany has shown the correlation of factors like age, blood glucose and blood lipids with CRP and fibrinogen [17]. In our study, age was correlated to the fibrinogen levels, with a marginal statistical significance (p=0.051).

Blood pressure was also correlated to these new risk markers. Especially, with CRP, this correlation was very strong [18]. In our study, hsCRP was significantly correlated only to SBP (r=0.21), but its correlation with SBP, DBP and pulse pressure all are known [18]. Fibrinogen was also elevated significantly with respect to blood pressure in our study (for SBP, r=0.24; for DBP, r=0.278; p<0.05). Different studies have also found this link between hypertension and the fibrinogen levels, which is probably due to increased viscosity [19].

Since our study was cross sectional, it was limited in its ability to find out the relative importance of the above factors in risk stratification. For that, prospective studies with a relatively larger patient population are needed.

CONCLUSION

In this study, the diabetic patients with ischaemic stroke were found to have significantly higher levels of hsCRP and fibrinogen. These variables also correlated significantly with the vascular risk markers like age, blood pressure and blood glucose. For homocysteine, the non-diabetic subset was found to have a higher level. The actual impact of these newer risk factors needs to be assessed by doing larger prospective studies. However, this study shows that these newer risk factors can be measured in diabetic patients for a better risk prediction. Also, the need for better blood pressure and blood sugar control has to be emphasized. Plasma homocysteine may be an independent risk factor in non-diabetic ischaemic stroke patients and its measurement as a screening test needs further studies.

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