ORIGINAL ARTICLE

A Study of Serum High Sensitivity C-Reactive Protein and Homocysteine Levels in Young Adults with Acute Ischemic Stroke

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ABSTRACT

Background: High sensitivity C-reactive protein (hsCRP) and homocysteine are considered important markers for cardiovascular and cerebrovascular diseases. Many studies have shown that high hsCRP and homocysteine levels are risk factors for ischemic stroke. Objective: Our objective is to investigate hsCRP and homocysteine levels in blood in young (49 years or below) and older (over 49 years) adults with ischemic stroke in a tertiary care hospital.

Material and Methods: We retrospectively studied forty acute ischemic stroke patients admitted at Owaisi Hospital and Research Centre, Hyderabad, for six months (June 2019 to December 2019). Patients were divided into younger and older adults and all underwent tests as per standard protocol for stroke.

Results: HsCRP level was elevated in 20 of 24 older stroke patients and in all young stroke patients ($p<0.05$). The mean hsCRP was significantly high in stroke patients overall (11.7mg/L).Fasting serum homocysteine level was elevated in 12 patients, mean value in them being 31.54 mg/dl and the difference was insignificant between younger and older patients.

Conclusions: Elevated hsCRP, fasting homocysteine levels are strongly associated with acute ischemic stroke. A higher proportion of young stroke patients are found to have elevated hsCRP levels compared to older adults, while there is no significant difference in fasting homocysteine levels between the same.

Key words: Ischemic Stroke, HsCRP, Homocysteine, Young adults, Indian population.

INTRODUCTION

Ischemic stroke is usually associated with atherosclerosis which is due to inflammation and injury in the arteries. There are many known inflammatory markers in blood, of which high sensitive C- Reactive Protein (hsCRP) is well known.[1, 2] It is a glycoprotein and is formed in the liver.[3-7] Elevated CRP levels were studied in young ischemic stroke patients and were found to increase mortality.[8] Hyperhomocysteinemia is considered a strong risk factor for ischemic stroke.[9] It causes vascular injury, which predisposes people to various diseases like stroke and coronary artery disease.10,11 Our aim is to compare the serum hsCRP (> 1mg/L) and homocysteine levels in young and older patients with ischemic stroke in Indian population at a tertiary centre.

MATERIAL AND METHODS

This is a retrospective observational study on the data of stroke patients treated at our hospital. Forty consecutive patients with acute ischemic stroke admitted between June 2019 and December 2019 in Department of Neurology, Owaisi Hospital and Research centre, Hyderabad, India were studied retrospectively. Stroke patients were taken into the study if the admission to the hospital was within 72 hours of onset of stroke. Patients with transient ischemic attack, hypoglycaemia, intra cerebral haemorrhage, subarachnoid haemorrhage were excluded. Patients were excluded from the study if they had any specific autoimmune diseases that can cause elevated serum CRP levels. A diagnosis of ischemic stroke was made on the basis of detailed history, examination, CT and MRI of brain. MRA of the brain, transthoracic echocardiography and Doppler of neck vessels were done in all patients. Additional tests were performed when required. We classified the strokes using TOAST classification into large artery disease, cardio embolic stroke, small vessel disease, stroke of other determined etiology, and stroke of undetermined etiology. [12] Blood tests including blood sugar, lipid profile, homocysteine levels, renal profile, and serum hsCRP were done. Tests for autoimmune disorders, procoagulant state and sickling tests were done if stroke subtype was not clear.

Measurement of serum hsCRP and homocysteine: Quantitative measurement of hsCRP was

done using VITROS 5.1 chemistry system in serum and normative data was taken from VITROS 5600 manual and literature. Levels < 1.0 mg/L were considered normal, 1.0-3.0 mg/L as moderate risk, and > 3.0 mg/L as high risk.[13] We considered hsCRP level of >1 mg/L as elevated and ≤ 1 mg/L as normal in this study. Fasting serum homocysteine was measured using chemiluminescence immunoassay (CLIA) method.

Statistical analysis: Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 16.0). Continuous variables were shown as mean ± SD, proportions were used for categorical variables, and chi-square test was used to study the association in proportions. After above analysis, p values less than 0.05 were taken as statistically significant.

RESULTS

We studied forty acute ischemic stroke patients during a period of six months from June 2019 to December 2019. Of them 26 patients were males and 14 were females. Other demographic data of patients is given in Table 1. None of the patients included in the study died. Young stroke patients (aged 49 years or below) were 16 in number and there were 24 older stroke patients (aged above 49 years).

The mean serum hsCRP level of study patients overall was 11.7 mg/L. The mean level was 12.9 mg/L in the 36 patients who had elevated level (above 1 mg/L) (Table 2). Serum hsCRP levels were found to be elevated in all young stroke patients and in 83% of older stroke patients which was statistically significant with p value less than 0.05 (Table 3). We observed a significant difference in the range of elevated hsCRP values among these two groups. Younger stroke patients showed serum hsCRP levels predominantly in the range of 1 to 10 mg/L. In contrast patients older than 49 years showed a wider range of hsCRP values from less than 1 to very high values of greater than 10 mg/L. Serum hsCRP greater than 10 mg/L were found in 8 older stroke patients (33%) whereas only 1 young stroke patient (6.2%) had such a high level. Comparison of hsCRP levels between males and females revealed a mean value of 15.5 mg/L in males and 4.59 mg/L in females which was not statistically significant. The prevalence of elevated hsCRP level was highest in patients with large artery atherosclerosis (100% patients) (Table 4) and next was small vessel disease (86% patients). Other stroke subtypes patients were very few in our study and so could not compare them with above two stroke subtypes.

Serum fasting homocysteine levels were elevated in 12 of the 40 study patients and the mean value in these 12 patients was 31.54 mg/dl. Mean level was 14.32 mg/dl in the group of 28 patients who had normal levels (i.e. <20 mg/dl) (Table 5). This difference was statistically very significant with a p value of less than 0.0001. Levels were elevated in 6 of 16 young stroke patients (37.5% of young stroke patients) and in 6 of 24 older stroke patients (25% of older stroke patients). Younger stroke patients aged up to 49 years showed a mean serum homocysteine level of 20.5 mg/dl and patients older than 49 years showed a mean value of 18.7 mg/dl. This difference was minimal and statistically insignificant (Table 6).

Table 1. Demographic parameters, vascular risk factors and other data of stroke patients.

Parameter	Young Stroke patients (n= 16)	Older stroke patients (n= 24)
Males	11	15 (35%)
Females	5	9
Age range (years)	35-49	50-82
Hypertension	7 (43.7%)	17 (70.8%)
Diabetes	5 (31.2%)	11 (45.8%)
High hscrp	16(100%)	20(83.3%)
Hyperhomocysteinemia	6 (37.5%)	6 (25%)
Deaths	0	0

Table 2. Serum hsCRP levels in total patients in the study irrespective of age.

HsCRP Level	mean ± SD (mg/L)
<1 mg/L (Normal) (n=4)	0.67 ± 0.14
>1 mg/L (Abnormal) (n=36)	12.92 ± 25.85
Overall Mean for total	11.70 ± 24.77

T value = 0.93, Df = 38, p>0.05; Statistically Not Significant

Table 3. Serum hsCRP levels distributed according to age into young adults and older adults.

Age distribution	HsCRP (> 1 mg/L)		
	<1 (Normal)	1-10 (Elevated)	>10 (High elevated)
<49 years	0	15	1
>49 years	4	12	8
Total	4	27	9

Chi square value = 8.51, P value < 0.05, Statistically significant
Correlation of HsCRP with Age- Correlation Coefficient (r) 0.36, P value <0.05

Table 4. Elevated hsCRP in stroke subtypes

Stroke subtype	Cases	Elevated* hsCRP (%)
Large artery atherosclerosis	16	16 (100)
Small artery disease	22	19 (86.3)
Cardio embolic stroke	1	1 (100)
Stroke of other determined etiology	0	0
Stroke of undetermined etiology	1	0

* Patient with Elevated Serum hsCRP (> 1 mg/L)

Table 5. Serum fasting homocysteine level in all study patients irrespective of age

Fasting Homocysteine (mg/dl)	Mean ± SD
<20 (Normal) (n=28)	14.32 ± 3.1
>20 (Abnormal) (n=12)	31.54 ± 7.16
Overall Mean	19.48 ± 9.23

T value = 10.72, Df = 38, p<0.0001; Statistically Significant

Table 6. Mean serum fasting homocysteine levels in young and older adults.

Age group	Fasting Homocysteine (mean ± SD) (mg/dl)
<49 years (n=16)	20.56 ± 10.76
>49 years (n=24)	18.76 ± 8.22
Overall Mean	19.48 ± 9.23

T value = 0.59, Df = 38, p>0.05; Not Statistically Significant

Table 7. Fasting homocysteine levels among stroke subtypes

Stroke subtype	Cases	Elevated* Homocysteine
Large artery atherosclerosis	16	8 (50)
Small artery disease	22	3 (13.6)
Cardio embolic stroke	1	1 (100)
Stroke of other determined etiology	0	0
Stroke of undetermined etiology	1	0

* Patient with Elevated Serum Homocysteinelevel (> 20 mg/L)

Comparison of homocysteine levels between males and females revealed a mean value of 19.7 mg/dl in males and 18.7 mg/dl in females which was not statistically significant. The prevalence of hyperhomocysteinemia was highest in stroke associated with large artery atherosclerosis (50% patients) and was very less in small vessel disease patients (only 14% patients). There was only one cardio embolic stroke patient in our study. Although he showed elevated level of serum homocysteine this subtype had not been compared due to the want of a larger number of patients (Table 7).

DISCUSSION

Inflammation and tissue damage in human body leads to production of acute phase reactants in liver and their subsequent release in blood. CRP is one of these substances.[14,15] CRP stimulates the endothelial cells to produce various adhesion molecules, such as intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin[16,17] which allow migration of mononuclear cells and T lymphocytes into the vessel wall and lead to atherosclerotic plaque formation. [18, 19] CRP also helps in releasing of superoxide anion and stimulation of tissue factor activity. [20] CRP is implicated in endothelial cell lysis, plaque erosion which are important in cardiovascular and cerebrovascular events.

This retrospective study included 40 patients of acute ischemic stroke. HsCRP was elevated in 90% of the ischemic stroke patients we studied. Our findings are consistent with a study by Rajput et al. whose study showed that 132 (88%) stroke patients had elevated CRP (CRP > 10 mg/L). [21] Di Napoli et al. from Italy showed in his study that 95 patients (74.2%) with ischemic stroke had elevated CRP levels (> 0.5 mg/L). [22] Elevated CRP (> 10 mg/L) in a study by Muir et.al. was noted in 96 out of the 228 (42.1%) ischemic stroke patients in the UK. [23] All patients of young stroke in our study were found to have elevated hsCRP while 83.3% of older stroke patients had hsCRP >1mg/L. Mean value of hsCRP in males was found to be 15.52 ± 30.06 while that in females was 4.59 ± 4.32, the difference however was statistically not significant (p value >0.05). Therefore, elevated serum hsCRP could be a prothrombotic factor in both males and females. Studies by Devraj et al. [24] and Wakugawa et al. [6] found that raised hsCRP level was significant only in men and not in women stroke patients. Muir et al. found no difference in elevated CRP (> 1 mg/L) levels in ischemic stroke patients based on gender. [23] The findings in our study correlate with findings of Muir et al. Recent studies revealed that CRP was associated with an increased risk of heart failure. [25] These and many other studies show that hsCRP is both risk factor and prognostic factor for cardio and cerebrovascular diseases. [7, 26, 27, 28] Our study demonstrated that high serum hsCRP level was significantly associated with stroke in all age groups, although more number of younger patients were found to have elevated levels than older patients. In this study, high hsCRP levels were associated with both large artery and small vessel stroke subtypes. We had only one patient with a cardio embolic stroke and therefore not considered for comparison.

Hyperhomocysteinemia is another entity considered to produce a procoagulable state in individuals. A fasting serum homocysteine of more than 20mg/dl is considered hyperhomocysteinemia. A study by Hao et al. showed that decreased serum levels of folate, vitamins B12 and B6, male sex, and urban life were significantly related to hyperhomocysteinemia in Chinese patients. [29] We found that 30% of the total stroke patients involved in our study had hyperhomocysteinemia. The mean homocysteine value was 31.54 ± 7.16 in twelve patients who had an elevated value and it was 14.32± 3.1 in 28 patients who had normal value below 20 mg/dl. When analysed, this was found to be statistically significant (p<0.0001).

Hyperhomocysteinemia was a finding more common (37.5%) in young adults as compared to older patients (25%), as seen by frequency analysis. We per-

formed multiple logistic regression analysis and derived a mean serum fasting homocysteine level of 20.56 ± 10.76 in young adults and 18.76 ± 8.22 in older adults. The difference in mean homocysteine values between these groups was statistically insignificant. A study by Gajbhare et al. showed that serum homocysteine levels were significantly high in young stroke patients. [30] In a study by Raheem SA, mean homocysteine levels were elevated in patients with infarcts. [31] In our study mild difference in mean serum fasting homocysteine levels was seen in male (19.79 ± 8.65) and female patients (18.92 ± 10.54), albeit not statistically significant ($p > 0.05$). In another Indian study, the difference in homocysteine levels with regard to sex were statistically not significant. [32] Results of our study are co-relating with some other studies done previously and confirm hyperhomocysteinemia as contributing factor in cerebrovascular disease.

CONCLUSION

Our study demonstrates that elevated serum hsCRP level is strongly associated with acute ischemic stroke and a higher proportion of young stroke (up to 49 years age) patients is found to have elevated levels compared to older adults. High serum fasting homocysteine level is associated with ischemic stroke, but there is no significant difference between young and older stroke patients. The results of this study give us a platform to establish interventions to reduce the risk factors of ischemic stroke. Our limitation has been smaller number of patients and further randomized control studies with larger number of young stroke patients would help establish the risk factors' role and benefit of interventions to control them.

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