HIGH SENSITIVITY C- REACTIVE PROTEIN LEVELS AND LIPID PROFILE IN ISCHEMIC AND HAEMORRHAGIC STROKE

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ABSTRACT

Introduction: Stroke is the neurological deficit of abrupt onset attributable to focal vascular cause and makes a considerable contribution to morbidity and mortality. High sensitivity C reactive protein (hsCRP) is an acute-phase reactant tends to increase at the onset of inflammation. Atherosclerosis, a major risk factor for cerebrovascular diseases involves inflammation which is triggered by dyslipidaemia. **Objective:** To estimate and compare levels of serum hs-CRP and lipid profile in patients with ischemic and haemorrhagic stroke. **Methods:** Present study comprised of 90 subjects, 30 ischemic stroke, 30 haemorrhagic stroke and 30 as apparently healthy control. Blood samples obtained within 24 hours of presentation were analysed for serum hsCRP and lipid profile. **Results:** In the present study Median age was 52 years, 52.5 years and 54 years in control, ischemic stroke and haemorrhagic stroke respectively. Hs-CRP levels were raised in ischemic and haemorrhagic stroke compared to normal control (F-value=96.78; $p<0.0001$). Total cholesterol, triglyceride and LDL- cholesterol levels were significantly raised while HDL- cholesterol levels were low in ischemic stroke and haemorrhagic stroke than control ($p<0.05$). **Conclusion:** Increased serum hs-CRP levels and dyslipidemia were observed in ischemic and haemorrhagic stroke. But serum hs-CRP cannot differentiate type of stroke.

Keywords: Ischemic stroke; Haemorrhagic stroke; hs-CRP; Lipid profile.

INTRODUCTION

Stroke is a clinical syndrome with rapidly developing loss of brain function(s) due to impairment in the blood supply to the brain because of blocked or burst blood vessel. This can occur due to ischemia caused by thrombosis or embolism or due to haemorrhage [1]. Recent studies have found that 7% of medical and 45% of neurological admissions were due to stroke with a fatality rate of 9% at hospital discharge and 20% at 28 days [2].

The diagnosis of stroke is based on history and clinical findings and the brain imaging is used to support the diagnosis. The clinical manifestations of stroke are variable because of complex brain anatomy and its vasculature. Stroke is classified on the basis of its aetiology as either ischemic or haemorrhagic. Despite the two types sharing similar risk profiles, they exhibit distinct molecular mechanisms in the acute phase [3].

Many studies have shown that stroke is having inflammatory pathology and the markers of inflammation have been proposed as new risk factors for stroke like elevated white blood cell count, endothelial nitric oxide synthase, intercellular adhesion molecule-1, lipoprotein associated Phospholipase A_2, Homocysteine, lipoprotein (a), small dense LDL, TNF, IL, D- dimer and serum amyloid A etc [4]. C-reactive protein (CRP) and dyslipidaemia are some additional markers to this growing list.

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CRP has been the most widely studied marker of inflammation. C- reactive protein (CRP) is an acute-phase reactant, produced in the liver, vascular smooth muscle cells and adipocytes. Synthesis and secretion of CRP tends to increase within hours of an acute injury or onset of inflammation [5]. CRP was discovered in 1930 by William Tillett and Thomas Francis of Rockefeller University, Oswald Avery and Maclyn McCarty, described that CRP as an 'acute-phase reactant' that was found to be increased in serum of patients during inflammation [6]. CRP consists of five identical, noncovalently associated □23-kDa protomers arranged symmetrically around a central pore. Each protomer has a recognition face with a phosphocholine binding site consisting of two coordinated calcium ions adjacent to a hydrophobic pocket [7].

MATERIAL AND METHODOLOGY

Study design: Cross sectional case control analytical study

Research place: The study was carried out in B.J. Govt. medical college Pune.

Ethics approval: The study design and protocol has been approved by the institutional ethical committee. Details and purpose of study was explained, and a signed informed consent was obtained as per the proforma from all the subjects or their legally responsible attendant.

Inclusion criteria: Subjects with age group 18 years to 60 years were chosen randomly irrespective of their sex amongst newly diagnosed patients of acute stroke ad-

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mitted within first 24 hours of stroke onset in medicine wards of Sassoon general hospital Pune. The diagnosis was confirmed by clinical signs, symptoms and CT scan or MRI brain.

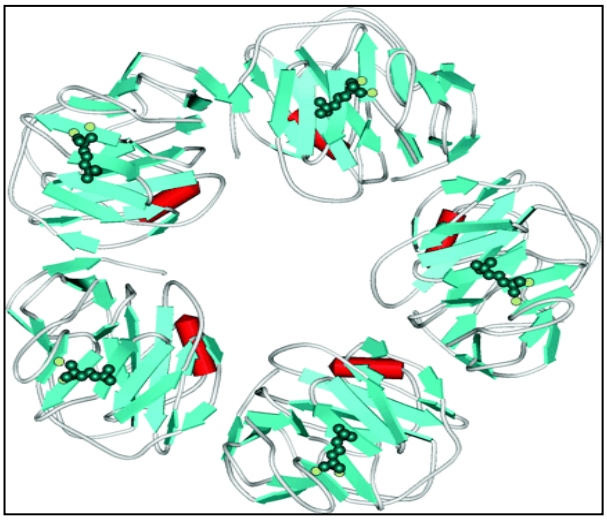

Fig 1: Pentameric structure of C- reactive protein [7]

Exclusion criteria: Stroke patients admitted after 24 hours of symptom onset and transient ischemic attack, cardiac diseases, cancer, infections, end stage renal disease, liver disease, uncontrolled diabetes mellitus and history of treatment of thyroid disease were excluded.

Sample size: Total 90 were included as per inclusion and exclusion criteria

Grouping: Based on radiological findings they were divided into two study groups, ischemic and haemorrhagic stroke, 30 cases each and compared with 30 age and sex matched apparently healthy controls.

Collection: About 5 ml of blood sample was withdrawn from the ante-cubital vein of each participant taking all aseptic precautions and blood was transferred to a clean dry sterile plain vacutainer. Blood specimen in plain tubes were allowed to clot for 30 minutes and then centrifuged and serum samples were used for estimation of hs-CRP and lipid profile (total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol).

Method: HsCRP was estimated by immunoturbidimetric assay using a buffer and latex particles coated with specific anti-human CRP antibodies as reagent[10].

Total Cholesterol by enzymatic cholesterol oxidase-peroxidase end-point method with lipid clearing system [11], triglyceride by glycerol phosphate oxidase and peroxidase; end point method [12], HDL-cholesterol by PEG precipitation method [13] and LDL-cholesterol using Friedewald equation [14].

Statistical analysis: Statistical software SPSS version 21.0 was used for data analysis. Continuous variables were presented as mean and standard deviation. Student's t-test was used to test the differences in biomarkers. The ANOVA test was used for significant difference between groups. A probability value of 0.05 was accepted as the level of statistical significance.

RESULTS

Demographic base line characteristics of the study groups were similar and shown in table 1.

Mean age of distribution among three groups is not significantly different ($p>0.5$). Similarly the gender distribution among three groups is not significantly different ($p>0.5$).

The mean hsCRP values in normal controls were 0.76 mg/L, in ischemic stroke 7.21mg/L and in haemorrhagic stroke 6.48mg/L. The rise in hsCRP was highly significant in ischemic and haemorrhagic stroke as compared to control group (p-value <0.001). While, there was no significant increase in hsCRP was found in ischemic stroke than haemorrhagic stroke (p-value>0.05).

Table 1 shows that there was a significant difference in the triglyceride levels in the ischemic stroke when compared to control group (p-value<0.05) but no significant difference was found between haemorrhagic and control group (p-value>0.05) and in between ischemic and haemorrhagic stroke group (p-value>0.05).

Total cholesterol levels show a significant difference in the study group (p-value<0.05) and significantly difference in levels was found in ischemic and haemorrhagic stroke when compared to controls (p-value<0.05), but no significant difference was found between ischemic and haemorrhagic stroke group (p-value>0.05).

There was statistically significant difference in the mean values of HDL-C in the study group (p-value<0.05). Significant difference in HDL-C levels was found between ischemic stroke and haemorrhagic stroke and ischemic stroke and control groups (p-value<0.05). But no significant difference was found when compared between haemorrhagic stroke and control (p-value>0.05).

VLDL-C were in haemorrhagic stroke group showing a statistically significant difference among study groups (p-value<0.05).

There was statistically significant difference in the mean values of LDL-C in the study group (p-value<0.05). Significant difference in LDL-C values was found in ischemic stroke and control group (p-value<0.05) but no statistical significant difference between haemorrhagic stroke and control group (p-value>0.05).

DISCUSSION

Stroke is the neurological deficit of abrupt onset attributable to focal vascular cause and makes a considerable contribution to morbidity and mortality [3]. Inflammatory theory in atherosclerosis suggests CRP is proinflammatory, pro-thrombotic and pro- atherosclerotic. It interacts with endothelial cells and stimulates the production of cytokines like IL-6, IL-1b, TNF, ET-1 and up -regulation of the expression of adhesion molecules (ICAM and VCAM). It directly increases the production of monocyte chemoattractant protein-1 and monocyte recruitment as well as activates the complement, promotes LDL uptake by macrophages and plaque formation which subsequently results in atherosclerosis and ultimately stroke [15].

30 diagnosed cases of each ischemic stroke and haemor-

Table 1: Comparison of lipid profile among three study groups

Parameter	Mean ± SD			F value of ANOVA	P value
	Control	Ischemic Stroke	Haemorrhagic Stroke		
Age (yrs)	50.07± 6.7	51.6 ± 8.42	48.63±10.28	0.894	.413
Gender M/F	17/13	18/12	19/11	Chi Square, 0.278	0.87 (NS)
hsCRP (mg/L)	0.76±0.41	7.21±2.37***	6.48±2.41$^{\$\$\$}$	96.78	<0.0001(HS)
TG (mg/dL)	100.3± 27.7	130.4 ±47.2*	115.9 ± 44.4	4.11	0.020 (S)
Total Cholesterol (mg/dL)	151.84±0.28	173.2±30.26*	165.8 ± 27.46$^{\$}$	5.09	0.008 (S)
HDL (mg/dL)	45.03 ± 7.7	39.37 ± 7.1*	44.47 ± 7.1$^{\#}$	5.4	0.006 (S)
LDL (mg/dL)	87.2±17.58	107.83±27.73*	98.40 ± 26.89	5.33	0.006 (S)
VLDL (mg/dL)	19.6 ± 5.5	26.00 ± 9.44*	22.93 ± 8.8	4.04	0.021 (S)

*Control vs. Ischemic stroke, $^{\$}$Control vs. Haemorrhagic Stroke, $^{\#}$Ischemic vs. Haemorrhagic Stroke, $^{*\$\#}$:Significant, $^{**\$\$\#\#}$: very significant $^{***\$\$\$\#\#\#}$: Highly significant, NS: Insignificant, S: Significant, HS: highly significant

rhagic stroke admitting in medicine ward of our institute within 24 hours of symptom onset were studied along with age and gender matched 30 healthy normal controls. High sensitivity C reactive protein and lipid profile were analyzed in all subjects.

The age distribution of the patients in this study was between 24 and 60 years. The risk of stroke increased with increasing age as maximum incidence was found in age group 51-60 years (66.7% in ischemic and 50% in haemorrhagic stroke) in the present study. These findings were in corroboration with studies by Mishra Talreja P, et al [16] and Chowdhury N et al [17].

In the study gender distribution of the patients in cases showed male preponderance. In ischemic stroke cases 43.3% were females and males were 56.7% with gender ratio of 1.5:1 while 40% females and 60% males were enrolled in haemorrhagic stroke group with gender ratio of 1.7:1.

In present study serum high sensitivity C reactive protein (hsCRP) levels were significantly increased in the stroke cases as compared to controls group (P-value <0.0001). It was also observed that there was a notable increase in ischemic stroke group (7.21±2.37mg/L) and haemorrhagic stroke (6.48±2.41mg/L) when compared with normal control (0.76 ±0.41mg/L) (F-value=96.78; p<0.0001) pointing towards underlying inflammatory pathology. The similar results were shown by Elkind MS V et al [18]. On intergroup comparison no significant difference was seen between ischemic and haemorrhagic stroke (p>0.05). Similar study by Roudbary SA et al [19] reported significant increase (P<0.0001) in hs-CRP levels in ischemic stroke than haemorrhagic stroke but Pandey et al [20] and Mishra PT et al [16] observed significant high levels of hs-CRP in haemorrhagic than ischemic stroke (P<0.001).

Andersson J. et al. first ever established the reference range for hsCRP for cerebrovascular risk assessment that hsCRP, divided into three groups, <1mg/L: Low Risk, 1 to 3 mg/L: Average Risk and >3 mg/L: High Risk, and is significantly associated with the risk of first -ever stroke. All these findings support serum hsCRP findings in present study [21].

Dyslipidaemia was associated with both types of stroke compared to control in present study. Mean serum total cholesterol levels were 151.84±20.20 mg/dl in control, 173.2±30.26 mg/dl in ischemic stroke and 165.8±27.46 mg/dl in haemorrhagic stroke group with a significant difference among the groups (F-value=5.09; p<0.05). Additionally, total cholesterol levels were significantly raised in ischemic stroke and haemorrhagic stroke than control (p<0.05) but no significant difference was there between haemorrhagic and ischemic stroke group. Mean values of triglyceride was significantly elevated in the ischemic stroke cases (130.4±47.2 mg/dl) compared to control group (100.3± 27.7mg/dl), (p<0.05) but there was no statistically significant difference observed in TG levels in haemorrhagic stroke (115.93± 44.47 mg/dl) compared to control and ischemic stroke group. These findings were in corroboration with study by Mahmood Asad et al [22].

Serum HDL-cholesterol is anti-atherogenic in nature and triggers the flow of cholesterol from peripheral cells to the liver and thus having a protective effect [23]. There is an inverse association between HDL-cholesterol and ischemic stroke in the present study. Mean serum HDL-C levels was low in ischemic stroke (39.37±7.19 mg/dl) when compared to haemorrhagic stroke (44.47±7.14 mg/dl) and controls (45.03± 7.70 mg/dl) with a significant difference of F-value= 5.4; p<0.05). However, statistically similar values of HDL-C were observed between haemorrhagic and control. Recently it has been observed that serum HDL-cholesterol levels decrease significantly at the time of acute ischemic stroke and it may be an acute phase reactant or nascent biomarker of acute stroke susceptibility [23]. There was a significant rise in mean serum LDL-C in ischemic stroke cases (107.83± 17.58 mg/dl) as compared to haemorrhagic stroke (98.40 ±26.89 mg/dl) group and control (87.2± 17.58 mg/dl). VLDL-C was analyzed only to calculate LDL-C by Friedewald's formula. These results were in accordance with other studies con-

ducted by Chowdhury S.R. et al [23] and Kun Han et al [24].

CONCLUSION

From the present study we concluded that hsCRP levels are increased in ischemic as well as haemorrhagic stroke suggesting inflammatory aetiology but cannot help differentiating the type of stroke. Total cholesterol, LDL cholesterol and triglyceride levels were significantly raised in stroke cases than control. Serum HDL-C levels were significantly decreased in ischemic stroke patients but not in haemorrhagic stroke when compared to control.

Limitations: The study was conducted on small sample size and observations were based on one time estimation of hsCRP and lipid profile rather serial measurements.

Conflict of Interest: Declared none

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