

ORIGINAL RESEARCH**Correlation Between IL6 and hsCRP (High Sensitive C-reactive Protein) with Ischaemic Stroke in Patients on Antidiabetic Therapy****¹Nibha, ²Shashi Kala Kumari, ³Ravi Kant, ⁴Jayendra Kumar, ⁵Vivek Sinha**¹Junior Resident, ³Assistant Professor, ⁵Professor & Head, Department of Biochemistry, Narayan Medical College and Hospital (NMCH), Jamuhar, Sasaram, Bihar, India²Assistant Professor, Department of Pharmacology, Narayan Medical College and Hospital (NMCH), Jamuhar, Sasaram, Bihar, India⁴Assistant Professor, Department of Neurosurgery, Anugrah Narayan Magadh Medical College and Hospital (ANMMCH), Gaya, Bihar, India**Correspondence:**

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Email: rkks09@gmail.com**ABSTRACT****Aim:** To study the correlation between IL6 and hsCRP (High sensitive C-reactive Protein) with Ischaemic stroke in patients on Antidiabetic therapy.**Materials and Methods:** We recruited 200 participants with Ischaemic stroke from both outpatient and in-patient. In this study patients were divided into two groups. Group A Ischaemic stroke with DM and group B with Ischaemic stroke but without DM. Blood was drawn with minimally traumatic venipuncture for measurement of serum inflammatory markers. Blood was centrifuged by 3,000 g for 15 min at 4 °C, and then aliquots were stored at -70 °C. Serum hs-CRP was measured by latex turbidimetric immunoassay with a sensitivity of 0.01 mg/L. Serum IL-6 were measured by enzyme-linked immunosorbent assay. With a sensitivity of 0.01 pg/mL. The detectable limit for Serum IL-6 was 0.01 pg/mL.**Results:** There were significant differences in the traditional risk factors such as age, the prevalence of hypertension, coronary heart disease, silent lacunar infarction (SLI) and WMLs. MoCA Scores or the using rate of antithrombotic drugs between the group 1 and group 2. ($P < 0.05$). Similarly, there were significant differences such as age, or the prevalence of alcohol intake, SLI and WML between the DM and NON-DM group ($P < 0.05$). We observed that serum hs-CRP and IL-6 levels (median: 7.94, and 9.34, respectively) in the group 1 were higher than in those (median: 4.31 and 6.68,) without DM group 2. Logistic regression analysis showed that after adjustment for age, sex, BMI, smoking, alcohol intake, hypertension, diabetes, hyperlipidemia, coronary heart disease, the use of antithrombotic drugs and the presence of SLI and WML, inflammatory marker level remained to be associated with DM. The adjusted ORs of hs-CRP and IL-6 were 1.98 (1.55-2.42) and 1.56 (1.33-1.86) in group 1 and 2.58 (1.79-3.36) and 1.69 (1.22-2.57) in group 2.**Keywords:** IL6, hsCRP (High Sensitive C-reactive Protein), Ischaemic Stroke, Antidiabetic Therapy.**INTRODUCTION**

Ischemic stroke is the most common cause of disability in elderly people (over 65 years of age) and the third most common cause of death in the world. In the course of the brain

damage, a number of biochemical mediators and inflammatory markers are released, ie cytokines, chemokines, pro-inflammatory enzymes and growth factors. A particularly rapid change in expression concerns pro-inflammatory cytokines.^{1,2} IL-6 is believed to be a cytokine of particular importance in the acute stroke phase. It is responsible for inducement of synthesis, mainly in the liver, of acute-phase proteins (APPs): C-reactive proteins (CRP) and fibrinogen.^{3,4} Many authors point out that the increase in the concentration of IL-6 in serum in the first day of stroke is associated with a deterioration in the functional status of patients⁵⁻⁷ and with a higher volume of ischemic lesions. It is also considered that IL-6 levels are a reliable prognostic factor in the acute phase of ischemic stroke.^{3,4} CRP level, as a reagent of an acute immune response stimulated by IL-6, may also increase in acute stroke phase, although they may also occur in response to a wide spectrum of systemic inflammatory conditions. According to many authors, the increase in hsCRP levels is associated with poor prognosis, whereas other researchers suggest that CRP is not a prognostic factor in ischemic stroke.^{5,6}

An increasing body of evidence shows that treatment with antidiabetic agents substantially reduces CRP concentrations. Although insulin appears to be the most potent agent, oral antidiabetic agents, such as biguanides (metformin), thiazolidinediones, and sulfonylureas, have also been shown to reduce CRP concentrations. However, the mechanism of this effect remains unclear. Clinical trials data suggest that CRP concentrations may be influenced only moderately by glycaemic control. After 6 months of intensive antidiabetic treatment, more than 50% of patients with type 2 DM maintained CRP concentrations of greater than 0.3 mg/dL (concentrations consistent with high risk), despite achieving glycaemic control. Furthermore, agents that produce comparable levels of glycaemic control have differential effects on CRP concentrations, suggesting that no direct association exists between glycaemic control and CRP concentrations.⁷ The aim of this study is to evaluate the correlation between IL6 and hsCRP (High sensitive C-reactive Protein) with ischaemic stroke in patients on Antidiabetic therapy.

MATERIALS AND METHODS

We recruited 200 participants with Ischaemic stroke from both outpatient and in-patient. In this study patients were divided into two groups. Group A Ischaemic stroke with DM and group B with Ischaemic stroke but without DM.

The data including age, gender, body weight, height and medical history of patients such as smoking, alcohol intake, heart disease, blood pressure, blood glucose, serum lipid and the use of antithrombotic drugs were recorded for analysis. Hypertension was defined as blood pressure $\geq 140/90$ mmHg on measurements taken on at least two occasions, or patients with a history of hypertension and using antihypertensive drug. Hyperlipidemia was defined as triglyceride level ≥ 1.7 mmol/L; low-density lipoprotein cholesterol level ≥ 3.4 mmol/L; total cholesterol level ≥ 5.72 mmol/L, or use of cholesterol-lowering therapy. Diabetes was defined as fasting blood-glucose ≥ 7.0 mol/L and/or postprandial blood sugar ≥ 11.1 mol/L, or use of antidiabetic therapy. Body mass index was defined as weight [kg]/height [m]². Smoking was defined as ever smoking but giving up and current smoking. Habitual alcohol intake was defined as alcohol drinking more than 20 g/day antiplatelet drugs and anticoagulant drugs are regarded as antithrombotic drugs. Montreal Cognitive Assessment Scores were used to assess cognitive function.

Blood was drawn with minimally traumatic venipuncture for measurement of serum inflammatory markers. Blood was centrifuged by 3,000 g for 15 min at 4 °C, and then aliquots were stored at -70 °C. Serum hs-CRP was measured by latex turbidimetric immunoassay with a sensitivity of 0.01 mg/L. Serum IL-6 was measured by enzyme-linked

immunosorbent assay with a sensitivity of 0.01 pg/mL. The detectable limit for Serum IL-6 was 0.01 pg/mL.

STATISTICAL ANALYSIS

Data was analyzed using SPSS 25.0. Measurement data was described as mean \pm standard deviation (SD). Enumeration data was described as number (%). *T*-test and one-way analysis of variance was used for comparisons of continuous variables. Kruskal-Wallis test followed by the Mann-Whitney *U* test were used for comparisons between groups. Multivariate logistic regression analyses were used for calculation of odds ratio, in which logarithmically transformed values of inflammatory markers were used. The results are shown as the odd ratios (OR) with 95% confidence interval (CI). Probability values were 2-tailed, and values of $P < 0.05$ were considered significant.

RESULTS

The baseline characteristics of the patients in this study are shown in Tables 1. There were significant differences in the traditional risk factors such as age, the prevalence of hypertension, coronary heart disease, silent lacunar infarction (SLI) and WMLs. MoCA Scores or the using rate of antithrombotic drugs between the group 1 and group 2. ($P < 0.05$, Table 1). Similarly, there were significant differences such as age, or the prevalence of alcohol intake, SLI and WML between the DM and NON-DM group ($P < 0.05$). We observed that serum hs-CRP and IL-6 levels (median: 7.94, and 9.34, respectively) in the group 1 were higher than in those (median: 4.31 and 6.68,) without DM group 2. [Table 1]. Correlations between Patients with ischemic stroke on antidiabetic therapy and inflammatory markers are summarized in Table 2. Logistic regression analysis showed that after adjustment for age, sex, BMI, smoking, alcohol intake, hypertension, diabetes, hyperlipidemia, coronary heart disease, the use of antithrombotic drugs and the presence of SLI and WML, inflammatory marker level remained to be associated with DM. The adjusted ORs of hs-CRP and IL-6 were 1.98 (1.55-2.42) and 1.56 (1.33-1.86) in group 1 and 2.58 (1.79-3.36) and 1.69 (1.22-2.57) in group 2.

Table 1: Comparison of baseline characteristics between group 1 and group 2

	DMwith Ischaemic stroke group	No DMwith Ischaemic stroke group	
Parameter	N=100	N=100	P
Age (years)	58.85 \pm 9.45	59.74 \pm 10.63	0.02
Male	60	52	0.55
BMI (kg/m²)	27.85 \pm 4.25	26.85 \pm 3.69	0.07
Smoking	43	47	0.52
Alcohol intake	53	49	0.36
Hypertension	67	40	0.001
HLP	43	37	0.65
CHD	59	42	0.03
SLI	71	31	0.001
Antithrombotic drugs	55	36	0.02
MoCA Score	25.11 \pm 0.36	26.87 \pm 0.31	0.007
hs-CRP [mg/L (M, Q1-Q3)]	7.94 (6.82-8.84)	4.31 (3.21-6.45)	0.000
IL-6 [pg/mL (M, Q1-Q3)]	9.34 (7.79-13.31)	6.68 (4.83-8.88)	0.000

Table 2: OR (95% CI) for DMwith Ischaemic stroke group andNo DMwith Ischaemic stroke group

Parameter	DMwith Ischaemic stroke group	No DMwith Ischaemic stroke group
	OR (95% CI)	OR (95% CI)
hs-CRP	1.98 (1.55-2.42)	2.58 (1.79-3.36)
IL-6	1.56 (1.33-1.86)	1.69 (1.22-2.57)

DISCUSSION

Inflammatory biomarkers serve as immune system health indicators, and in vascular research, elevated levels support evidence of ongoing disease processes that can up-regulate atherosclerosis or induce pro-thrombotic states.⁸ Cytokine profiles produce snapshots of dynamic and complex immune system responses to the milieu of acute and chronic stimuli. High sensitivity C-reactive protein (hsCRP) is one of the most investigated cytokines in cardiovascular research and has been found to predict ischemic stroke in some, but not all populations. Interleukin-6 (IL6) is a pro-inflammatory cytokine similarly associated with increased vascular risk, but it is also paradoxically linked to anti-inflammatory molecules through complex auto-inhibitory feedback mechanisms.⁹⁻¹¹

In our study, hypertension, SLI, WML were associated with higher incidence of DM, and thus were strong risk factors of DM. Our results were consistent with previous studies. Shams et al¹². observed that patients with Ischaemic stroke were significantly aged, had hypertension, and had lower cognitive function. A number of studies have shown that age is an independent risk factor of Ischaemic stroke. Vernooij et al.¹³ found that the incidence of Ischaemic stroke increased with age from 17.8% in persons aged 60-69 years to 38.3% in those over 80 years. There were significant differences in MoCA Scores and using antithrombotic drugs between the DM group and NON-DM group. Several studies reported significant association between the presence of ischaemic stroke and IL6.^{14,15} All the Ischaemic stroke patients had various degrees of WML and different numbers of SLI. Gregoire et al.¹⁶ found that Ischaemic stroke were more numerous and prevalent in antiplatelet users who developed symptomatic ICH compared with matched antiplatelet-users who did not develop ICH. This data suggested a potential role for Ischaemic stroke as a risk factor for antiplatelet-associated ICH. The relationship between alcohol intake and Ischaemic stroke needed to be further studied. Our study showed that the levels of hs-CRP and IL-6 in group 1 were significantly higher than those in group 2. The regression analysis showed that inflammatory factors such as hs-CRP and IL-6 were the independent risk factors of Ischaemic stroke. Previous studies found significant levels of hs-CRP and IL-6.¹⁷ Hoshi et al.¹⁸ found that IL-6 could result in increase of hs-CRP as an important cytokine in inflammation, it can be a predictor of SLI. Koh et al.¹⁹ found that the levels of MMP-9 and hs-CRP were significantly higher in patients with DM than in those without.

CONCLUSION

We concluded that Ischaemic stroke were closely related with the age, prevalence of hypertension, SLI, WML, MoCA Score, the using rate of antithrombotic drugs, levels of hs-CRP and IL-6 suggesting a role of inflammatory processes in DM.

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