

TEMPORAL PROFILE OF SERUM LEVELS OF IL-6 IN ACUTE ISCHEMIC STROKE AND ITS RELATIONSHIP WITH STROKE SEVERITY AND OUTCOME IN INDIAN POPULATION

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ABSTRACT

Introduction: Acute ischemic stroke is characterized by sudden loss of blood supply to brain leading to cerebral ischemia and neurological damage. The ischemic event causes neuroinflammation and release of inflammatory cytokines from immune cells of brain tissues. One of such cytokine is the Interleukin-6 which is released from the neuroglia cells of the brain after an acute ischemic attack.

Purpose of the study: To estimate the serum levels of IL-6 serially at various time intervals in acute ischemic stroke patients and comparing them with healthy control; to evaluate the association of baseline serum IL-6 levels with stroke severity and outcome.

Materials and method: In 33 cases of acute ischemic stroke IL-6 was measured in serum by ELISA on the day of admission, day seventh, one month, and at third month. Severity of stroke was assessed clinically using national institute of health stroke scale (NIHSS) at day one and radiologically by infarct volume on diffusion weighted imaging (DWI) of MRI within initial seven days. The short term outcome was assessed by NIHSS at seventh day and long term outcome by modified rankin scale (MRS) at 1 month and three months. IL-6 levels were also measured in age and sex matched 60 healthy controls.

Results: Serum levels of IL-6 were significantly high among cases than healthy controls at all stages. Baseline IL-6 levels in cases showed a significant positive correlation with stroke severity and outcome.

Conclusion: Baseline levels of IL-6 correlates with stroke severity and predicts worse outcome on short term and long term.

KEY WORDS: Ischemic stroke, IL-6, NIHSS, MRS, Infarct volume.

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Online Access and Article Informtaion

Quick Response code



DOI: 10.16965/ijims.2017.121

International Journal of Integrative Medical Sciences

www.imedsciences.com

Received: 11-12-2017

Accepted: 08-01-2018

Reviewed: 11-12-2017

Published: 31-01-2018

Source of Funding: Self

Conflicts of interest: None

BACKGROUND

There are many evidences that inflammation and immune response play an important role in the outcome of ischemic stroke patients, and

they have been associated to larger brain damage [1]. After the acute event of stroke, the proinflammatory cytokine IL-6 is rapidly released by activated microglia, astrocytes and neurons

and invading cells of the immune system [2].

IL-6 is a multifunctional cytokine produced by various types of cells and regulates the immune response, hematopoiesis, the acute phase response and inflammation [3] and is related to mortality and severity of acute diseases in the Emergency Department [4].

Moreover, in numerous previous studies of ischemic stroke it has been found that increased levels of pro-inflammatory cytokines were related to a greater extent of cerebral infarct and poorer clinical outcome [5,6].

The aim of our study was to measure the inflammatory cytokine IL-6 serially in acute ischemic stroke patients and to see their association with stroke severity and outcome.

MATERIALS AND METHODS

Study group: The study was conducted in Biochemistry Department of Maulana Azad Medical College and Department of Neurology, G.B.Pant Hospital, New Delhi after the approval by institutional ethics committee. It was a case control study. Written informed consent was taken from the cases and controls. A total of 33 consecutive acute ischemic stroke patients (CT/MRI proved) admitted in neurology ward of G.B.Pant Hospital, New Delhi were included in study group based on inclusion criteria. Briefly, the exclusion criteria were patients presenting with CT/MRI proven hemorrhagic stroke, with transient ischemic attack only (deficit resolving completely within 24 hours), fever at the onset or history of fever in the recent past (1 week prior to stroke), history of rheumatological disease, autoimmune disease, or any kind of acute or chronic infection, immunosuppressive therapy like corticosteroids, regular analgesic intake, severely impaired renal and hepatic functions. Age and sex matched 60 healthy controls were recruited for the study. The controls were not having any past history of diabetes, hypertension, stroke, transient ischemic stroke, dyslipidemia, coronary artery disease and no intake of non steroidal anti inflammatory drugs in past 1 week.

A detailed history and clinical evaluation was carried out; baseline NIHSS score was used for clinical severity. Radiological severity was assessed by infarct volume on DWI of MRI scan [7].

Assessment of patients and serial blood sampling was done at day one (admission day), day seven, one month and three months following admission using a defined protocol. The short term outcome was assessed by NIHSS at seventh day. To see the long term outcome, patients were clinically assessed at 1 month and 3 months using MRS. We used TOAST criteria [8] to classify the subtypes of ischemic stroke in cases. Lateralization of the stroke was determined by clinical findings and imaging results. Location of the stroke, supra- or infratentorial respectively, was determined by radiological findings. Hypertension was defined in the study population as systolic blood pressure greater than 140mm Hg and diastolic blood pressure greater than 90mm Hg. Patients with fasting plasma glucose more than 126mg/dl and postprandial more than 200mg/dl were labelled as diabetics. Dyslipidemia was defined as fasting serum cholesterol more than 200mg/dl, triglycerides more than 150mg/dl, and HDL less than 40mg/dl in males and less than 50mg/dl in females. A person was labelled dyslipidemic if any one of the above criteria was present.

Blood collection and serum separation: The blood for serum analysis was first collected on day 1 following admission in fasting stage, subsequently at day 7, 1 month, 3 months. The first collection of sample was done after median gap of 4 days after stroke onset (range 0-7 days, mean 3.84 days). 5 ml of fasting venous blood sample was collected in plain vial and stored for 45 to 60 min at room temperature prior to centrifugation at 1,400 rpm for 12 min. Serum was separated and stored at -80 °C till further analysis.

Measurement of IL-6 levels in serum: Quantitative estimation of IL-6 was done by using Human IL-6 ELISA kit (Gen-Probe Diaclone SAS France).

Statistical Analysis: Epi Info™ software was used to perform statistical analyses. Continuous data was summarized as mean, median and range (minimum, maximum). Student t test was used to compare the means. Categorical data was summarized as frequencies and percentages. Since the IL-6 levels were not normally distributed, we logarithmically transformed the values to obtain normal distribution. Association

of peak levels of serum IL-6 with the severity of stroke was seen by using Pearsons correlation. Univariate and multivariate linear regression was done for outcome analysis because it was best suitable for sample size of this study. Results were considered significant when the p-value was < 0.05.

RESULTS

Baseline characteristics: A cohort of 33 consecutive acute ischemic stroke patients between the age range of 18 to 75 years formed the initial study group. All of them presented within 7 days of onset of stroke. Biochemical analysis of serum obtained on day of admission was done for all 33 patients. Out of these 33 patients, long-term follow up (up to 3 month) was successfully completed in 23 patients (6 patients died and 4 were lost to follow up). All demographic plus clinical data and the stroke characteristics are shown in table 1 and 2 respectively.

Assessment of stroke severity: Mean \pm SD for NIHSS score at admission was 8.42 ± 5.84 and the median was 6.0. On the basis of day 1 NIHSS score 57.57% cases had mild stroke (NIHSS score 0 – 7), 24.24% had moderate stroke (NIHSS score 8 – 13) and 18.18% had severe stroke (NIHSS score > 14). The mean \pm SD for infarct volume was 38.18 ± 55.55 ml and the median was 8.4ml. On the basis of infarct volume 66.67% cases had mild stroke, 12.12% had moderate stroke and 21.21% had severe stroke.

Serum levels of IL-6 in cases Vs control: The mean value for IL-6 at admission day was 21.91 ± 30.75 pg/ml with a median of 10.41pg/ml, which was significantly higher as compared to controls whose mean was 1.04 ± 1.12 pg/ml, and median was 0.79 pg/ml. Subsequently, IL-6 levels as measured on day seven (12.80 ± 21.18), one month (6.18 ± 14.21) and three month (2.75 ± 2.54) were also significantly higher than control. There was consistent decline in serum values of IL-6 from baseline to day 7, 1 month and 3 months in cases. [Figure no.1]

Baseline levels of IL-6 and stroke severity: The higher baseline IL-6 level showed a significant positive correlation with the NIHSS score at day 1 ($r^2 = 0.18$; $p = 0.01$) and with the infarct volume on DWI ($r^2 = 0.17$; $p = 0.01$) [figure no.2 and 3

respectively].

Baseline levels of IL-6 and stroke outcome: It was found that that higher baseline levels of IL-6 were significantly correlated with the poorer short term outcome as measured by NIHSS Score on day 7 following admission ($r^2 = 0.20$; $P = 0.01$) [figure no.4]. When this correlation was extended for long term outcome it was found that IL-6 correlated with poorer long term outcome both at 1 month ($r^2 = 0.19$; $P = 0.01$) and 3 months ($r^2 = 0.15$; $P = 0.04$) as measured by MRS [figure no. 5 and 6 respectively]. In order to find out whether the baseline IL-6 independently predict stroke outcome, we performed a univariate followed by multivariate analysis for predictor of stroke outcome. We entered the variables age, sex, hypertension, diabetes, dyslipidemia, baseline NIHSS score, infarct volume on DWI and IL-6 levels day 1 for predicting the short term and long term outcome. The variables which were significant were then entered into a multivariate analysis to see whether they independently predict the outcome. We found that baseline IL-6 levels was independent predictors of short term outcome as assessed by NIHSS at day 7 ($P = 0.04$); also for long term outcome as measured by MRS at one month ($P = 0.02$) and three months ($P = 0.04$). Baseline levels of IL-6 were significantly higher ($P = 0.04$) in those patients who died than those who survived [figure no.7].

Table 1: Demographic and clinical characteristics of patients and controls.

Characteristics	Stroke cases	Control
N	33	60
Age (Mean \pm SD)	53.69 \pm 12.98	50.48 \pm 8.67
Female	15	26
Male	18	34
NIHSS on admission (Mean \pm SD)	8.42 \pm 5.84	-
Infarct volume (ml) (Mean \pm SD)	38.18 \pm 55.55	-
Risk factors		
Dyslipidemia	30 (90.9%)	-
DM	20 (60.6%)	-
HTN	20 (60.6%)	-
Anaemia	12 (36.4%)	-
Smoking	11 (33.3%)	-
Tobacco	10 (30.3%)	-
RHD	8 (24.2%)	-
CAD	7 (21.2%)	-
Alcohol	6 (18.2%)	-

Table 2: Stroke characteristics (n=33).

TOAST category	N
Large vessel disease	14 (42.42%)
Small vessel disease	7 (21.21%)
Cardio embolic	8 (24.24%)
Undetermined	4 (12.12%)
Determined aetiology	0 (0%)
Location of infarct	
Supratentorial	27(81.81%)
Infratentorial	6(18.18%)

Fig. 1: Trend line showing the changes in mean value of serum levels of IL-6 over a time period from day 1, day 7, 1 month and 3 months following admission.

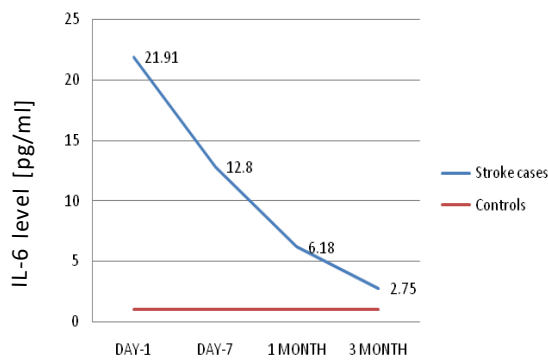


Fig. 2: Scatter plot showing significant correlation between IL-6 and NIHSS at day 1.

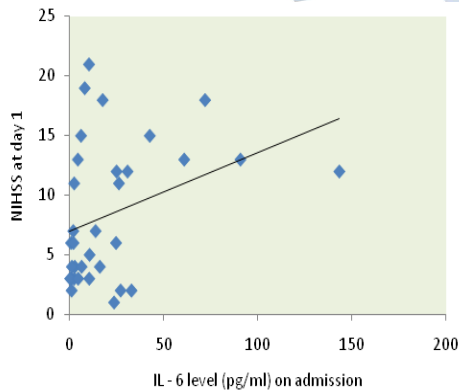


Fig. 3: Scatter plot showing significant correlation between IL-6 and infarct volume.

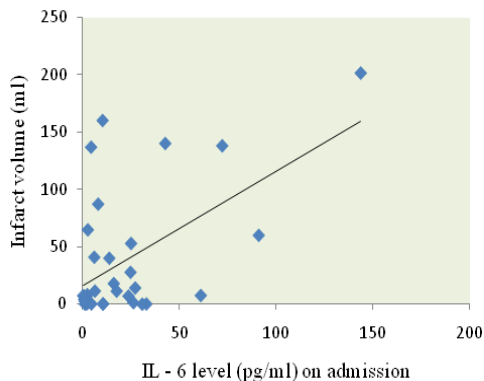


Fig. 4: Scatter plot showing significant correlation between IL-6 on day of admission and NIHSS score at day 7.

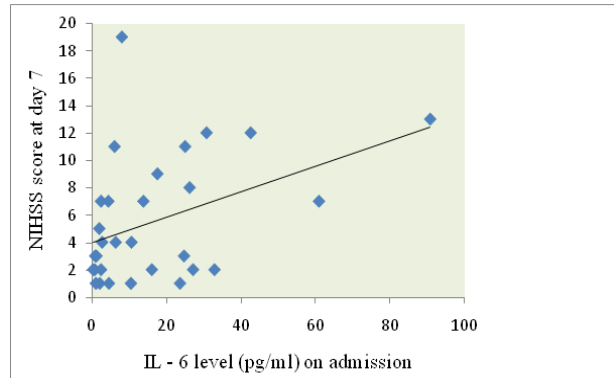


Fig. 5: Scatter plot showing significant correlation between IL-6 on day of admission and MRS score at 1 month.

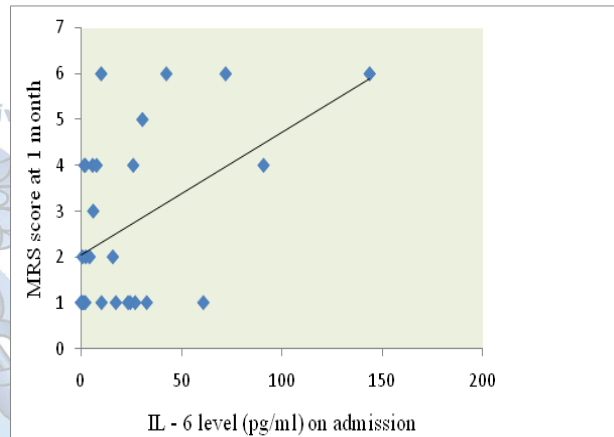


Fig. 6: Scatter plot showing significant correlation between IL-6 on day of admission and MRS score at 3 months.

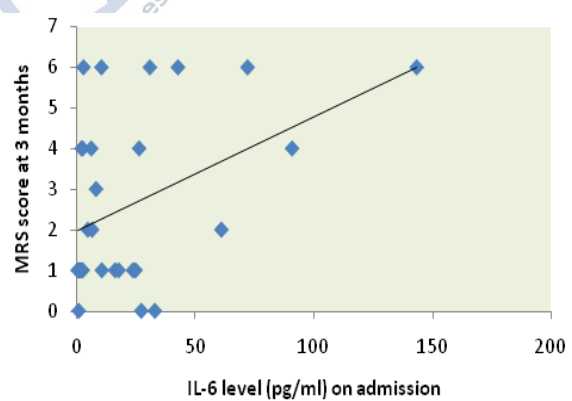
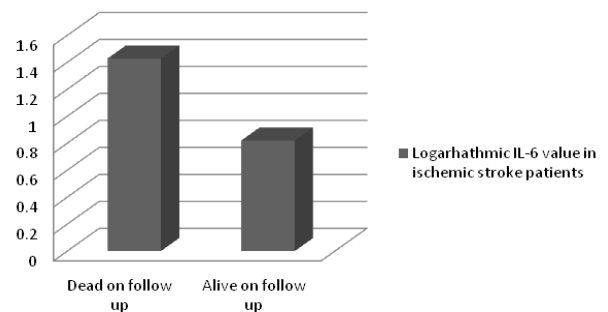


Fig. 7: Log IL-6 value in ischemic stroke patients (Dead vs Alive)



DISCUSSION

In the recent years the extensive research is suggestive of that inflammation plays a crucial role in stroke pathophysiology by the release of various cytokines and acute phase proteins. But the exact role of these cytokines and their effect in acute phase and subsequently in late phase and how they does affect outcome of stroke is still a matter of debate. Since inflammation generates the formation of edema and can promote apoptosis, inflammatory biomarkers are potentially useful for prognosis and as prospective targets for neuroprotective therapies [9]. Although none of the cytokines that activate in the brain following stroke are CNS specific, careful investigation has confirmed that the elevation in plasma levels of these signaling molecules is owing to their production in the CNS and not from peripheral blood cells [10]. The aim of this study was to measure the serum IL-6 serially in acute ischemic stroke patients and to see the correlation of it's baseline levels with stroke severity and outcome.

In the present study, the mean value for IL-6 at admission day was 21.91 ± 30.75 pg/ml with a median of 10.41pg/ml, which was significantly higher as compared to healthy controls whose mean was 1.04 ± 1.12 pg/ml, and median was 0.79 pg/ml. Our results were in accordance with the finding of Inimioara Mihaela Cojocaru et al. [11].

The timing of sampling is important because with the evolution of stroke, these values show temporal variations. As evidenced by Perini et al [12] and Kim et al [13], the values start to rise in 24 hours and remain high for the next 7 days and there after it tapers and comes to normal in 6 months. We could also show that IL-6 levels at admission, day 7, one month and three months were significantly higher than control. Also, the values consistently declined over time period thereby suggesting subsiding of inflammation in post stroke period, but were still higher than the healthy controls that was consistent with the findings of Rallidis et al. [14].

Our study demonstrated a significant positive correlation between baseline serum IL-6 levels and stroke severity as determined by NIHSS day 1 and infarct volume on DWI. These findings

were in agreement with the results of Smith et al [1], who reported that peak plasma IL-6 concentrations were significantly correlated with the stroke severity at 5 to 7 days and with infarct volume. Orion D et al [15] found that elevated levels of IL-6 in acute stroke patients were correlated with a larger infarct volume. Vila et al. [6] showed a strong positive relationship of IL-6 levels in plasma and cerebrospinal fluid to early neurological worsening in patients in the first 24 h after stroke onset.

Further, significant correlation of baseline levels of IL-6 was seen with the short term outcome (NIHSS at day 7) and with long term outcome (MRS at 1 month and 3 month respectively). Again this observation was consistent with the Smith et al. [1] Finally, when univariate and multivariate analysis was done to see the predictive value of baseline IL-6 level in stroke outcome, it was observed that IL-6 emerged as independent predictor of outcome.

We could also show that patients who died due to ischemic stroke had more severe stroke and higher levels of IL-6 as compared to patients who survived. Although small in number, this data suggested that higher baseline IL-6 may predict mortality in ischemic stroke. So, based on these finding it is reasonable to consider the IL-6 as a prognostic indicator for the development of clinical complications following acute ischemic events.

CONCLUSION

Serum levels of IL-6 were significantly higher in ischemic stroke patients than in healthy control. IL-6 showed a significant correlation with clinical and radiological severity of stroke, and was able to predict the poor outcome. The findings of this study supports the consideration of serum IL-6 as a potentially useful inflammatory biomarker for prognosis of acute ischemic stroke.

Limitations: Total number of ischemic stroke patients was less; however we took almost double number of control to compare the IL-6 values. IL-6 was measured with a median gap of 4 days after the onset of acute attack but this had not affected our results as the IL-6 levels start to rise in 24 hrs and remain high for the next 7 days.

Funding source: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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How to cite this article: Saroj Choudhary, Debashish Chowdhury, T.K.Mishra, Sarita Agarwal. TEMPORAL PROFILE OF SERUM LEVELS OF IL-6 IN ACUTE ISCHEMIC STROKE AND ITS RELATIONSHIP WITH STROKE SEVERITY AND OUTCOME IN INDIAN POPULATION. *Int J Intg Med Sci* 2018;5(1):555-560. DOI: 10.16965/ijims.2017.121