DOI: 10.7860/JCDR/2018/32132.11700

Original Article

Biochemistry Section

Correlation of Serum Nitric Oxide with Haematological and Biochemical Parameters in Acute Ischaemic Stroke Patients

PRITI CHATURVEDI[1], VINIT MEHROTRA[2], YOGESH SAXENA[3], SOUMEN MANNA[4]

ABSTRACT

Introduction: Nitric oxide (NO), different haematological and biochemical parameters, play an important role in the pathogenesis of Acute Ischaemic Stroke (AIS) (<24 hour).

Aim: To establish a correlation between NO with haematological and biochemical parameters in AIS patients.

Materials and Methods: A hospital based, cross-sectional study was done in 50 patients of AIS and 25 healthy controls. Serum NO level was measured by ELISA. Complete lipid profile, Random Blood Sugar (RBS), serum Blood Urea Nitrogen (BUN), creatinine and haemogram were assessed by automated devices. Pearson's correlation coefficients were analysed to look for the relationship between NO with biochemical and haematological parameters. The p-value was considered significant if $p<0.05$.

Results: Statistically highly significant ($p<0.001$) elevation in mean serum NO levels were observed in cases (32.06 µmol/L) as compared to controls (24.41 µmol/L). NO was positively correlated with elevated levels of Triacylglycerol (TAG), T-Chol, Low Density Lipoprotein (LDL-C), VLDL-C, RBS, and negatively correlated with High Density Lipoprotein (HDL-C), BUN, serum creatinine, Total Leukocyte Count (TLC), ESR, but none of the correlation coefficient was found to be statistically significant.

Conclusion: Elevated NO and derangement of biochemical and haematological parameters may be linked to pathogenesis of AIS.

Keywords: Acute stroke, Erythrocyte sedimentation rate, Lipoprotein cholesterol, Total leukocyte count

INTRODUCTION

The World Health Organization (WHO) definition of stroke is rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin [1,2]. Ischaemic strokes accounts for majority of strokes which occur due to interrupted blood supply to a brain area [3].

The NO is a gaseous molecule that can easily cross biological membranes and plays an important role in the pathogenesis of AIS (<24 hour). Cerebral ischaemia leads to multiple changes in NO signaling as well as NO content of brain [4]. NO may have some positive (neuroprotective) or, negative (neurodestructive) effects depending on the stage of stroke and isoform of NO produced during the stroke [5,6].

The NO derived from iNOS appears to contribute to neurotoxicity after ischaemic stroke, as animal knockout models have smaller infarcts than their wild type counterparts [7]. An increase in NO above basal levels secondary to iNOS occurs 12-24 hour after Middle Cerebral Artery (MCA) occlusion and contributes to neurotoxicity, with iNOS inhibition leading to reduced infarct volume [8].

Apart from NO, haematological parameters such as polymorphs and other types of WBCs had also been implicated in pathogenesis of stroke [9-12]. Many studies had also highlighted that various haematological and biochemical parameters like blood glucose, White Blood Cell (WBC), Erythrocyte Sedimentation Rate (ESR) alteration had prognostic significance in AIS [13,14]. Some of the studies had also shown that elevated WBC, ESR, creatinine was independent predictor of 30 days case fatality in patient with first time AIS [15,16].

Both NO and LDL has been associated with brain infarction. Studies [17,18] have also established the increased sequestration of leukocytes into the ischaemic area in next 4-6 hours of ischaemia of brain with the endogenous NO affecting the sequestration in vasculature. Since, both NO and biochemical/haematological parameters are involved in the pathophysiology of ischaemic stroke. The present study attempts to see the interaction of the above parameters in the stroke cohort.

MATERIALS AND METHODS

This hospital based, cross-sectional, observational study was conducted in Himalayan Institute of Medical Sciences (HIMS), Dehradun, India, over a period of 12 months (August 2009 to July 2010). Fifty patients of AIS, attending the indoor and outpatient departments of Medicine at HIMS were enrolled in this study as cases and 25 were healthy individuals who acted as controls. Age matched healthy controls were enrolled from subjects coming for routine health checkup and healthy blood donors.

Considering the limited study period and limited financial budget, to ensure adequate strength of study and its outcome a case to healthy comparative control ratio of 2:1 was considered. Based on previous studies and considering a mean difference of serum NO of 4.50 and the pooled Standard Deviation (SD) of 10.6, a sample size of 43 AIS cases was calculated [6,7]. With the 10% attrition the figure was rounded up to 50. With the case and control ratio of 2:1, 25 healthy controls were recruited for the study.

All patients included in the study had a confirmed clinical diagnosis of ischaemic stroke supported by immediate neuroimaging (CT/MRI). Stroke was defined according to WHO guidelines [1]. The time of onset of the stroke was defined as the time when the patient or observer first became aware of the symptoms.

All adult patients (55-70 years of age) suffering from ischaemic stroke, which is less than 24 hours duration were included in this study. The exclusion criteria were as follows: i) Stroke associated with myocardial infarction; ii) Stroke associated with Central Nervous System (CNS) infection; iii) Stroke in patients using nitrate drug; iv) Previous history of stroke.

After inclusion, each participant was subjected to thorough history taking and detailed clinical examinations {physical and systemic examination with emphasis on neurological examination and evaluation of Glasgow Coma Scale (GCS) score. After taking written

informed consent, serum NO, complete haemogram including ESR, complete lipid profile, serum BUN, creatinine, and RBS were evaluated from all participants.

Collection of Samples

Blood samples were collected at the time of admission. Three milliliters (mL) of blood was drawn from the antecubital vein in a plain vacutainer for the estimation of serum NO, RBS, BUN, urea and creatinine and 2 mL of blood was taken in Ethylenediaminetetraacetic Acid (EDTA) vacutainer for haemogram. After 12 hours of overnight fasting, 2 mL of blood was collected in a plain vacutainer for the estimation of lipid profile.

Estimation of serum NO was done by Enzyme-Linked Immunosorbent Assay (ELISA) technique with NO colorimetric assay kit using Griess reagent (BioVision Research Products, USA). Estimation of serum total cholesterol, HDL cholesterol, triglycerides, glucose, BUN and creatinine were done on SYNCHRON CX9, automated chemistry analyser (Beckman Coulter, USA). Serum LDL cholesterol and Very Low-Density Lipoprotein (VLDL) cholesterol were calculated by using Friedewalds formula [19]. Although, previous reports suggest that Friedewald's formula underestimates LDL-C only when value of triglyceride is very high, the equation remains one of the best in routine use [20]. Estimation of haemoglobin, total leukocyte count and differential leukocyte count were done on the automated haematology cell counter "MS-9". All the haematological and biochemical investigations were carried out in the laboratories of Himalayan Institute of Medical Sciences Hospital, Dehradun.

STATISTICAL ANALYSIS

Demographic data was stratified by gender to assess its association with AIS by Chi-square test. Difference between the case and control subjects were analysed by the unpaired t-test for demographic, biochemical parameters, haematological parameters and NO. Finally, the Pearson's correlation coefficients were analysed to look for the relationship between the NO and biochemical and haematological parameters among the acute ischaemic stroke patients.

The p-value was considered significant if $p<0.05$, highly significant if $p<0.01$ and very highly significant if $p<0.001$. All the data were expressed as mean±standard deviation (SD).

RESULTS

Number of cases and controls, mean age and sex are shown in [Table/Fig-1]. The difference between the mean age of controls and cases was statistically insignificant.

Parameter	Controls (n=25)	Cases (n=50)	p-value
Age in years (mean±SD)	53.64±5.62	57.18±10.64	0.064#
Sex (male/female)	18/7	32/18	0.07*
Hypertension	-	20 (40%)	-
Diabetes mellitus	-	17 (34%)	-
Smoking	7 (28%)	24 (48%)	-

[Table/Fig-1]: Comparison of baseline characteristics of controls and cases.
#unpaired "t" test, *chi-square test, p>0.05-non-significant (ns), p<0.05-significant

Among controls, the mean value and range of NO was 24.41 µmol/L and 14.94-32.31 µmol/L respectively. Among cases, the mean value and range of NO was 32.06 µmol/L and 17.55-43.70 µmol/L respectively. On comparison, statistically very highly significant elevation in mean serum NO level was observed in cases as compared to controls ($p<0.001$).

Comparison of serum biochemical profile among controls and cases are given in [Table/Fig-2]. Pearson's correlation matrix composed showed levels of NO were positively correlated with TAG, Total Cholesterol (T-Chol), LDL-C, VLDL-C, RBS and negatively correlated with HDL-C, BUN and serum creatinine, but none of the correlation coefficient was found to be statistically significant in the cohort. On the other hand, a statistically highly significant positive correlation of BUN with the serum creatinine ($r=0.541$, $p<0.01$) was found. The levels of blood glucose were correlated positively with the T-Chol and LDL-C levels and the relation was statistically very highly significant [Table/Fig-3].

Parameter	Status	Mean±SD	n	Mean Difference	p-value
TAG (mg/dL)	Controls	128.68±22.05	25	24.32	0.001
	Cases	153±36.41	50		
T-Chol (mg/dL)	Controls	185.88±32.87	25	31.58	0.001
	Cases	217.46±44.57	50		
LDL-C (mg/dL)	Controls	115.14±32.14	25	31.53	0.001
	Cases	146.68±42.08	50		
VLDL-C (mg/dL)	Controls	25.73±4.41	25	4.86	0.001
	Cases	30.60±7.28	50		
HDL-C (mg/dL)	Controls	45.00±7.70	25	4.82	0.012
	Cases	40.18±6.45	50		
RBS (mg/dL)	Controls	118.32±21.12	25	24.70	0.001
	Cases	143.03±37.81	50		
BUN (mg/dL)	Controls	15.8±3.58	25	4.24	0.001
	Cases	20.04±6.60	50		
Creatinine (mg/dL)	Controls	0.89±0.17	25	0.20	0.014
	Cases	1.10±0.50	50		

[Table/Fig-2]: Comparison of biochemical parameters (Mean±SD) among controls and cases.
unpaired "t" test, p>0.05-non significant, p<0.05-significant, p<0.01-highly significant, p<0.001-very highly significant.
TAG: Triacylglycerol; T-Chol: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; RBS: Random blood sugar; BUN: Blood urea nitrogen

Comparison of different haematological parameters between cases and controls has been shown in [Table/Fig-4]. Pearson's correlation matrix showed levels of NO were negatively correlated with the Hb, TLC, platelet and ESR; however none of the correlation coefficient was found to be statistically significant in the cohort. TLC was found to be positively correlated with the ESR and the relation was statistically very highly significant ($r=0.655$, $p<0.001$) [Table/Fig-5].

DISCUSSION

Cerebrovascular disease or stroke is one of the major cause of mortality and disability worldwide [21,22]. Several studies have established cellular and molecular pathophysiological processes in acute ischaemic stroke, which are complex [23,24]. Among the many players of stroke, NO and various haematological parameters were implicated in pathogenesis of stroke [5,10,11,25].

This study was planned to evaluate the serum levels of NO and other biochemical and haematological parameters in patients of ischaemic stroke of less than 24 hours duration and to find out a correlation between them.

The mean age of our stroke patients was 57.18±10.64 years which was relatively younger than those seen in the Western studies. In the Indian subcontinent, stroke happens nearly a decade earlier than West [26]. A total of 35 (70%) of the patients were in age group 41-60 years, 14 (28%) of the patients were aged more than 60 years and only 1 (2%) patient was less than 40 years of age. The gender distribution showed an M:F ratio of 1.78:1. Present study was comparable to other Indian studies on stroke patients where greater preponderance was seen among males [26,27].

In the present study, hypertensive and diabetic population was higher as compared to a similar study done by Aygul R et al., which may be due to increasing number of diabetic and hypertensive population in India [28].

	NO	TAG	T-Chol	LDL-C	VLDL-C	HDL-C	RBS	BUN	CREA
NO	1	0.118	0.247	0.253	0.118	-0.096	0.208	-0.091	-0.032
TAG		1	0.218	0.139	1.00***	- 0.453*	0.218	0.109	0.042
T-Chol			1	0.992***	0.218	0.214	0.624***	- 0.146	- 0.005
LDL-C				1	0.138	0.175	0.645***	- 0.167	- 0.016
VLDL-C					1	- 0.453*	- 0.125	0.109	0.042
HDL-C						1	0.240	- 0.019	0.041
RBS							1	- 0.013	0.111
BUN								1	0.541**
CREA									1

[Table/Fig-3]: Pearson's correlation among biochemical parameters in cerebral ischaemic patients.
*p<0.05-Significant, **p<0.01-Highly significant, ***p<0.001-Very highly significant
NO: Nitric oxide; TAG: Triacylglycerol; T-Chol: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; RBS: Random blood sugar; BUN: Blood urea nitrogen; CREA: Creatinine

Parameter	Status	Mean±SD	n	Mean difference	p-value
Hb (mg/dL)	Controls	13.42±1.17	25	0.59	0.07
	Cases	12.83±1.62	50		
TLC (/mm^3)	Controls	8492.56±1178.47	25	1547.64	0.001
	Cases	10040.20±2717.04	50		
Neutrophil (%)	Controls	72.68±4.99	25	4.8	0.001
	Cases	77.48±6.71	50		
Lymphocyte (%)	Controls	25.72±5.35	25	4.32	0.003
	Cases	21.40±6.39	50		
Eosinophil (%)	Controls	1.56±0.91	25	0.06	0.814
	Cases	1.62±1.24	50		
Platelet (105/ mm^3)	Controls	2.55±0.57	25	0.278	0.057
	Cases	2.27±0.59	50		
ESR (mm first hour)	Controls	10.36±2.36	25	19.64	0.000
	Cases	30.00±14.46	50		

[Table/Fig-4]: Comparison of haematological parameters (Mean±SD) among controls and cases.
unpaired "t" test, p>0.05-non significant, p<0.05-significant, p<0.01-highly significant, p<0.001-very highly significant.
Hb: Haemoglobin; TLC: Total leukocyte count; ESR: Erythrocyte sedimentation rate

	NO	Hb	TLC	Platelet	ESR
NO	1	-0.108	-0.269	-0.085	-0.004
Hb		1	-0.028	-0.444*	-0.409*
TLC			1	0.035	0.655***
Platelet				1	0.208
ESR					1

[Table/Fig-5]: Pearson's correlation of NO with GCS and haematological parameters in cerebral ischaemic patients.
*p<0.05-significant, **p<0.01-highly significant, ***p<0.001-very highly significant
NO: Nitric oxide; Hb: Haemoglobin; TLC: Total leukocyte count; ESR: Erythrocyte sedimentation rate

Serum NO levels were very highly elevated in cases as compared to controls in present study. Experimental evidence by Malinski T et al., had shown that AIS induced by occlusion of Middle Cerebral Artery (MCA) of rats resulted in increased brain NO production [29]. Concomitant with changes in brain NO levels, serum and CSF NO levels were also found to be significantly higher in various studies [6,28,30]. Contrary to the above studies, Shibata M et al., showed a decrease brain NO level, after production of global ischaemia by occluding the common carotid artery of rat [31]. Similarly blood NO level was found to be low in various other studies also [25,32]. In ischaemic stroke, hypoxia and hypercapnia may be the triggering factors for NOS enzyme as evidenced by cell culture study [33]. Because of this it is now widely believed that NO production is enhanced at all stages of cerebral ischaemia [34].

We had found out statistically highly significant level of TAG, T-Chol, LDL-C while low level of HDL-C was present in cases as compared to controls. Present findings were in agreement with other study on Indian population, where significant increase in the LDL-C level and a significant decrease in the HDL-C level were observed [35]. Contrary to present findings, a study had also shown lipid lowering effect of acute ischaemic stroke [36], the mechanism of lipid lowering effect is unclear. Consistent with many other studies [13,37,38], significant increase in blood glucose level in the present study may be due to stress response after AIS as suggested by Murros K et al., [39].

Analysis of haematological parameters showed a statistically significant increase in TLC and ESR in AIS patients than controls. These findings were in agreement with previous studies where alterations of WBC, ESR, polymorphs were reported [40,41]. Increase in ESR values observed in their study was, at least in part, a consequence of the acute phase response to the ischaemic stroke event as suggested previously [42]. A high WBC count could be pinpointing to a vast immune system complexity in strokes patients. In addition, previous studies reported that WBC particularly polymorphs participate in the acute phase of ischaemic brain injury [10,43]. The probable effects of WBC and polymorph in the pathogenesis of ischaemic stroke patients include restriction of cerebral blood flow due to vessels plugging, exacerbation of parenchymal injury via hydrolytic enzymes, free oxygen radical production, and initiation of thrombosis [14].

LIMITATION

The present study is a single center, hospital-based study on a smaller sample hence the strength of relation of NO and other factors for AIS cannot be expanded to the regional population. A larger population-based study is needed to establish the relation of NO to covariates of AIS in the regional community.

CONCLUSION

Elevation in serum levels of RBS, TAG, T-Chol, LDL-C and VLDL-C while low levels of HDL-C were observed in patients of AIS. These changes constitute important risk factors for cerebral ischaemia.

Significant elevation in mean serum NO levels in patients of AIS was most probably due to the induction of NOS by the ischaemic condition which contributes to subsequent neuronal damage.

The present study findings suggest that haematological derangement and oxidative stress in AIS may be the result of imbalance in oxidant/antioxidant homeostasis.

REFERENCES

[1] The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA project principal investigators. J Clin Epidemiol. 1988;41(2):105-14.
[2] Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the american heart association/american stroke association. Stroke. 2013;44(7):2064-89.
[3] Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Heart disease and stroke statistics-2010 update: a report from the american heart association. Circulation. 2010;121(7):e46-e215.
[4] Terpolilli NA, Moskowitz MA, Plesnila N. Nitric oxide: considerations for the treatment of ischemic stroke. J Cereb Blood Flow Metab. 2012;32(7):1332-46.
[5] Iadecola C. Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci. 1997;20(3):132-39.
[6] Castillo J, Rama R, Davalos A. Nitric oxide-related brain damage in acute ischemic stroke. Stroke. 2000;31(4):852-57.
[7] Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME. Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci.1997;17(23):9157-64.
[8] Iadecola C, Zhang F, Xu X. Inhibition of inducible nitric oxide synthase ameliorates cerebral ischemic damage. Am J Physiol. 1995;268(1 Pt 2):R286-92.
[9] Prentice RL, Szatrowski TP, Kato H, Mason MW. Leukocyte counts and cerebrovascular disease. J Chronic Dis. 1982;35(9):703-14.
[10] Ernst E, Matrai A, Paulsen F. Leukocyte rheology in recent stroke. Stroke. 1987;18(1):59-62.
[11] Mercuri M, Ciuffetti G, Robinson M, Toole J. Blood cell rheology in acute cerebral infarction. Stroke. 1989;20(7):959-62.
[12] Rodrigues SF, Granger DN. Leukocyte-mediated tissue injury in ischemic stroke. Curr Med Chem. 2014;21(19):2130-37.
[13] Woo E, Chan YW, Yu YL, Huang CY. Admission glucose level in relation to mortality and morbidity outcome in 252 stroke patients. Stroke. 1988;19(2):185-91.
[14] Kochanek PM, Hallenbeck JM. Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. Stroke. 1992;23(9):1367-79.
[15] Bhatia RS, Garg RK, Gaur SP, Kar AM, Shukla R, Agarwal A, et al. Predictive value of routine hematological and biochemical parameters on 30-day fatality in acute stroke. Neurol India. 2004;52(2):220-23.
[16] Furlan JC, Vergouwen MD, Fang J, Silver FL. White blood cell count is an independent predictor of outcomes after acute ischaemic stroke. Eur J Neurol. 2014;21(2):215-22.
[17] Yilmaz G, Granger DN. Leukocyte recruitment and ischemic brain injury. Neuromolecular Med. 2010;12(2):193-204.
[18] Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol. 2007;184(1-2):53-68.
[19] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
[20] Jun KR, Park HI, Chun S, Park H, Min WK. Effects of total cholesterol and triglyceride on the percentage difference between the low-density lipoprotein cholesterol concentration measured directly and calculated using the friedewald formula. Clin Chem Lab Med. 2008;46(3):371-75.
[21] Johnson NB, Hayes LD, Brown K, Hoo EC, Ethier KA, Centers for Disease C. CDC national health report: leading causes of morbidity and mortality and associated behavioral risk and protective factors-United States, 2005-2013. MMWR Suppl. 2014;63(4):3-27.
[22] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):e442.
[23] Woodruff TM, Thundyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. Mol Neurodegener. 2011;6(1):11.
[24] Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. Int J Stroke. 2012;7(5):378-85.
[25] Rashid PA, Whitehurst A, Lawson N, Bath PM. Plasma nitric oxide (nitrate/nitrite) levels in acute stroke and their relationship with severity and outcome. J Stroke Cerebrovasc Dis. 2003;12(2):82-87.
[26] Anand K, Chowdhury D, Singh KB, Pandav CS, Kapoor SK. Estimation of mortality and morbidity due to strokes in India. Neuroepidemiology. 2001;20(3):208-11.
[27] Nagaraja D, Gururaj G, Girish N, Panda S, Roy AK, Sarma GR, et al. Feasibility study of stroke surveillance: data from Bangalore, India. Indian J Med Res. 2009;130(4):396-403.
[28] Aygul R, Kotan D, Demirbas F, Ulvi H, Deniz O. Plasma oxidants and antioxidants in acute ischaemic stroke. J Int Med Res. 2006;34(4):413-18.
[29] Malinski T, Bailey F, Zhang ZG, Chopp M. Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. J Cereb Blood Flow Metab. 1993;13(3):355-58.
[30] El Kossi MM, Zakhary MM. Oxidative stress in the context of acute cerebrovascular stroke. Stroke. 2000;31(8):1889-92.
[31] Shibata M, Araki N, Hamada J, Sasaki T, Shimazu K, Fukuuchi Y. Brain nitrite production during global ischemia and reperfusion: an in vivo microdialysis study. Brain Res. 1996;734(1-2):86-90.
[32] Cano CP, Bermudez VP, Atencio HE, Medina MT, Anilsa A, Souki A, et al. Increased serum malondialdehyde and decreased nitric oxide within 24 hours of thrombotic stroke onset. Am J Ther. 2003;10(6):473-76.
[33] Fathi AR, Yang C, Bakhtian KD, Meng QI, Lonser RR, Pluta RM. Carbon dioxide influence on nitric oxide production in endothelial cells and astrocytes: cellular mechanisms. Brain Res. 2011;1386:50-57.
[34] Kader A, Frazzini VI, Solomon RA, Trifiletti RR. Nitric oxide production during focal cerebral ischemia in rats. Stroke. 1993;24(11):1709-16.
[35] Narang AP, Verma I, Kaur S, Narang A, Gupta S, Avasthi G. Homocysteine--risk factor for ischemic stroke? Indian J Physiol Pharmacol. 2009;53(1):34-38.
[36] Zitnanova I, Siarnik P, Kollar B, Chomova M, Pazderova P, Andrezalova L, et al. Oxidative stress markers and their dynamic changes in patients after acute ischemic stroke. Oxid Med Cell Longev. 2016;2016:9761697.
[37] Christensen H, Boysen G. Blood glucose increases early after stroke onset: a study on serial measurements of blood glucose in acute stroke. Eur J Neurol. 2002;9(3):297-301.
[38] Southerland AM, Johnston KC. Considering hyperglycemia and thrombolysis in the Stroke Hyperglycemia Insulin Network Effort (SHINE) trial. Ann N Y Acad Sci. 2012;1268:72-78.
[39] Murros K, Fogelholm R, Kettunen S, Vuorela AL, Valve J. Blood glucose, glycosylated haemoglobin, and outcome of ischemic brain infarction. J Neurol Sci. 1992;111(1):59-64.
[40] Elkind MS, Cheng J, Boden-Albala B, Paik MC, Sacco RL, Northern Manhattan Stroke S. Elevated white blood cell count and carotid plaque thickness: the northern manhattan stroke study. Stroke. 2001;32(4):842-49.
[41] Chitsaz A, Tolou-Ghamari Z, Ashtari F. Preliminary evaluations related to the ranges of hematological and biochemical variables in hospitalized patients with stroke. Int J Prev Med. 2013;4(Suppl 2):S347-52.
[42] Zaremba J, Skrobanski P, Losy J. Acute ischaemic stroke increases the erythrocyte sedimentation rate, which correlates with early brain damage. Folia Morphol (Warsz). 2004;63(4):373-76.
[43] Hallenbeck JM, Dutka AJ, Tanishima T, Kochanek PM, Kumaroo KK, Thompson CB, et al. Polymorphonuclear leukocyte accumulation in brain regions with low blood flow during the early postischemic period. Stroke. 1986;17(2):246-53.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.
2. Professor, Department of Biochemistry, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.
3. Associate Professor, Department of Physiology, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.
4. Assistant Professor, Department of Physiology, Swami Rama Himalayan University, Dehradun, Uttarakhand, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Soumen Manna,
Assistant Professor, Department of Physiology, Swami Rama Himalayan University, Jolly Grant,
Dehradun-248016, Uttarakhand, India.
E-mail: drsoumen.manna@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Sep 01, 2017**
Date of Peer Review: **Oct 17, 2017**
Date of Acceptance: **Mar 29, 2018**
Date of Publishing: **Jun 01, 2018**