Correlation of Serum Nitric Oxide (NO) with Glasgow Coma Scale (GCS) in Acute Ischemic Stroke Patient: A Study in North India

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Abstract Nitric oxide (NO) is one of the key players in the pathogenesis of ischemic stroke. Limited reports are available about the serum level of NO and their correlation with Glasgow Coma Scale (GCS) score in acute (<24 h) ischemic stroke (AIS) patients. A hospital based, cross sectional study was done in North Indian population to find out correlation of serum NO and GCS in AIS patients. 50 patients of AIS and 25 healthy controls were chosen for the study. Serum NO level was measured by ELISA and GCS scores were assessed by a neurologist. Pearson correlation coefficients were analyzed to look for the relationship between NO and GCS. Statistically highly significant elevation in mean serum NO level was observed in cases as compared to controls ($p < 0.01$). A negative correlation of NO levels with neurological score of GCS $r(48) = -0.144$, $p > 0.05$ was seen. This indicates oxidative stress in acute ischemic stroke may be the result of imbalance in oxidant/antioxidant homeostasis.

Keywords Nitric oxide (NO) · Glasgow Coma Scale (GCS) score · Oxidant/antioxidant · Acute ischemic stroke

Introduction

Cerebrovascular disease or, stroke is a medical emergency condition that may lead to death. Stroke is the fifth leading causes of death worldwide and one of the most common causes of impairment in Western world [1]. Although there is decline in the relative rate of stroke death from 2001 to 2011 by 35.1 to 21.2%, still each year, approximate 8 million people have a new or recurrent stroke (ischemic or hemorrhagic). In the United States according to 2011 data, stroke caused approximately 1 of every 20 deaths [2].

The annual incidence rates (AIR) of stroke in metro cities of India like Mumbai (AIR of 148 per 100,000 persons) and Kolkata (AIR of 123.15 per 100,000 persons) are higher than that in United States (107 per 100,000 per year) and European countries (61–111 per 100,000 per year) [3–5].

Nitric oxide (NO) plays an important role in the pathogenesis of cerebral ischemic stroke [6]. Large amounts of NO are produced from neuron, glial cells, endothelial cell and macrophages in central nervous system. NO is physiologically produced by two calcium-calmodulin dependent nitric oxide synthase (NOS) enzymes. NOS isoforms present in *endothelial cells* (eNOS), and *neurons* (nNOS) produce small (nanomolar) amounts of NO for short periods in response to transient increases in intracellular calcium. NO generated by eNOS and nNOS is essential for the control of *cerebral blood flow*, whereas nNOS derived NO also functions as a neurotransmitter and is involved in *synaptic plasticity*, modulation of neuroendocrine functions, memory formation and

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behavioral activity [7, 8]. Third nitric oxide synthase isoform, inducible or immunological NOS (iNOS) is not a constitutive enzyme like eNOS and nNOS and is only produced after cytokine stimulation of neutrophils resulting from local ischaemia [9].

The isoform of NOS determines whether it functions as neuroprotective (eNOS) or, neurodestructive (nNOS and iNOS) [10]. Irrespective of the type of NO, the effects of NO in ischemic stroke are controversial [11].

Several studies reported an increase [12–18] while others reported a decline [19–22] in NO levels in acute ischemic stroke. Most of the studies have been done in animals while few in humans.

Very few studies had reported the correlation between blood NO levels in acute ischemic stroke and Glasgow Coma Scale (GCS) score [23]. Therefore the present study was undertaken to evaluate the NO levels in ischemic stroke of less than 24 h duration and to establish a correlation between the NO levels and the GCS score.

Subjects and Methods

A hospital based, cross sectional, observational, case control study was conducted in Himalayan Institute of Medical Sciences (HIMS), Dehradun, Uttarakhand, India, over a period of 12 months. The study population was derived from the patients attending the indoor and outpatient Departments of Medicine at HIMS. The study group included 75 subjects out of which 50 were patients of ischemic stroke and 25 were healthy individuals who acted as controls. The patients included in the study satisfied the following criteria:

Inclusion Criteria

1. All adult patients more than 18 years age, suffering from ischemic stroke.
2. Ischemic stroke of less than 24 h duration.

Exclusion Criteria

1. Stroke associated with myocardial infarction.
2. Stroke associated with CNS infection.
3. Stroke of more than 24 h duration.
4. Stroke in patients using nitrate drug.

All patients included in the study had a confirmed clinical diagnosis of ischemic stroke supported by immediate neuroimaging (CT/MRI). The time of onset of the stroke was defined as the time when the patient or observer first became aware of the symptoms.

After inclusion, each patient was subjected to a detailed clinical evaluation.

A detailed history was obtained including personal and family history, history of neurological disorders, and treatment history. After obtaining informed written consent from the subjects or their guardians, a thorough general and systemic examination was performed on all patients and findings were entered in a predesigned proforma. The study also included 25 healthy subjects of comparative age groups, who served as controls. These mainly included subjects coming for routine health checkup and healthy blood donors.

Besides serum nitric oxide levels, complete hemogram, including ESR were evaluated from all participants.

Collection of Samples

At the time of admission, 3 ml of blood was drawn by venepuncture from the antecubital vein in a plain vacutainer for the estimation of serum nitric oxide, blood sugar, BUN and creatinine and 2 ml of blood was taken in EDTA vacutainer for hemogram. After 12 h 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 ELISA technique with nitric oxide colorimetric assay kit (BioVision Research Products, USA), using Griess reagent. Estimation of serum total cholesterol, HDL cholesterol, triglycerides, glucose, BUN and creatinine were done on SYNCHRON CX9, Automated Chemistry Analyzer of Beckman Coulter, USA. Serum LDL cholesterol and VLDL cholesterol were calculated by using Friedewalds formula. Estimation of hemoglobin, total leukocyte count and differential leukocyte count were done on the automated hematology cell counter “MS-9”. Glasgow Coma Scale scores were assessed by a neurologist.

Statistical Analysis

Demographic data was stratified by gender and subjected to analysis to assess the effect of biochemical parameters on the cohort. Difference between the case and control subjects were analyzed by the unpaired “t” test between both male and females for all the bio-chemical parameters, hematological parameters, NO and neurological assessment (GCS). Finally the Pearson correlation coefficients were analyzed to look for the relationship between the NO and GCS with bio-chemical and hematological parameters among the acute cerebral ischemic 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 $\pm$ standard deviation.

Results

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

Statistically very highly significant elevation in mean serum NO level was observed in cases as compared to controls. GCS score was lower in cases as compared to controls; the difference was statistically very highly significant (Table 2).

On comparison among males (cases and control), statistically highly significant elevation in mean serum NO levels was observed in cases as compared to controls. GCS score was lower in cases as compared to controls; the difference was statistically very highly significant (Table 3).

On comparison among females (cases and control), statistically significant elevation in mean serum NO levels was observed in cases as compared to controls. GCS score was lower in cases as compared to controls; the difference was statistically very highly significant (Table 4).

Pearson correlation matrix composed of biochemical and hematological profile in subjects with acute cerebral ischemia showed a negative correlation of NO levels with neurological score of GCS $r(48) = -0.144$, $p > 0.05$. 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 $r(48) = 0.655$, $p < 0.001$ (Table 5).

Discussions

Stroke is a common cause of death in many countries and is a major cause of severe physical disability, particularly in the middle-aged and elderly population. The pathophysiological processes in acute ischemic stroke are extremely complex and involve pathological permeability of the blood–brain barrier, energy failure, loss of cell ion

homeostasis, acidosis, increased intracellular calcium and free radical mediated toxicity, which has been described in number of recent reviews [24–26]. Several mechanisms protect lipids and other biomolecules from free radical damage in vivo; however, despite numerous defenses, the brain is vulnerable to oxidative stress resulting from ischemia and reperfusion. Furthermore, excitotoxic injury stimulates superoxide and nitric oxide production, leading to the formation of highly reactive products, including peroxynitrite and hydroxyl radicals, which are capable of damaging lipids, proteins and DNA.

The present study was planned to evaluate the serum levels of NO in patients of ischemic stroke of less than 24 h duration and to correlate between the NO levels and GCS score.

In the present study the mean age of the cases was 57.18 ± 10.64 years (32 males and 18 females) while the mean age of the controls was 53.64 ± 5.62 years (18 males and 7 females). The difference was statistically insignificant.

In the present study, among cases, 40% were hypertensive while the percentage was higher in studies done by El Kossi and Zakhary [16] and Aygul et al. [17]. In our study among cases, 48% were smokers. This is in accordance with the study of Aygul et al. [17], who reported 47% smokers among cases. The percentage of diabetics in our study was higher (34%) as compared to the study of Aygul et al. [17]. This may be because of increasing number of diabetics in India.

In the present study serum NO levels were elevated in cases as compared to controls and the difference was statistically very highly significant. The level of significance in our study is in agreement with other studies [16, 17]. In contrast, Cano et al. [22] and Rashid et al. [21] reported significantly decreased NO levels in cases as compared to controls.

In animal study also, elevated plasma nitrate/nitrite level is seen, when acute ischemic stroke is produced by middle cerebral artery occlusion in rats [13]. Kader et al. [14] reported that a sharp transient increase in the activity of nitric oxide synthase occurs during cerebral ischemia in

Table 1 Comparison of baseline characteristics (Mean $\pm$ SD) of controls and cases

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

* $p > 0.05$ —non significant (ns), $p < 0.05$ —significant, $p < 0.01$ —highly significant, $p < 0.001$ —very highly significant

Table 2 Comparison of nitric oxide and GCS (Mean $\pm$ SD) among controls and cases

Parameter	Status	Mean $\pm$ SD	n	Mean difference	Significance* <i>p</i> value
NO ($\mu\text{mol/L}$)	Controls	24.41 $\pm$ 6.41	25	7.64	0.000
	Cases	32.06 $\pm$ 5.86	50		
GCS	Controls	15.00 $\pm$ 0.00	25	4.94	0.000
	Cases	10.06 $\pm$ 3.08	50		

* $p > 0.05$ —non significant, $p < 0.05$ —significant, $p < 0.01$ —highly significant, $p < 0.001$ —very highly significant

Table 3 Comparison of nitric oxide and GCS (Mean $\pm$ SD) among male controls and cases

Parameter	Status	Mean $\pm$ SD	n	Mean difference	Significance* <i>p</i> value
NO ($\mu\text{mol/L}$)	Controls	25.21 $\pm$ 5.69	18	6.26	0.001
	Cases	31.47 $\pm$ 6.31	32		
GCS	Controls	15.00 $\pm$ 0.00	18	4.78	0.000
	Cases	10.21 $\pm$ 2.63	32		

* $p > 0.05$ —non significant, $p < 0.05$ —significant, $p < 0.01$ —highly significant, $p < 0.001$ —very highly significant

Table 4 Comparison of nitric oxide and GCS (Mean $\pm$ SD) among female controls and cases

Parameter	Status	Mean $\pm$ SD	n	Mean difference	Significance* <i>p</i> value
NO ($\mu\text{mol/L}$)	Controls	22.38 $\pm$ 7.80	7	10.70	0.010
	Cases	33.12 $\pm$ 4.60	18		
GCS	Controls	15 $\pm$ 0.00	7	5.22	0.000
	Cases	9.78 $\pm$ 3.82	18		

* $p > 0.05$ —non significant, $p < 0.05$ —significant, $p < 0.01$ —highly significant, $p < 0.001$ —very highly significant

Table 5 Pearson correlation of NO with GCS and hematological parameters in cerebral ischemic patients

	NO	GCS	Hb	TLC	Platelet	ESR
NO	1	−0.144	−0.108	−0.269	−0.085	−0.004
GCS		1	−0.242	−0.046	−0.077	0.017
Hb			1	−0.028	−0.444*	−0.409*
TLC				1	0.035	0.655***
Platelet					1	0.208
ESR						1

* $p < 0.05$ —significant, ** $p < 0.01$ —highly significant, *** $p < 0.001$ —very highly significant

rats, which leads to a burst in nitric oxide production and activation of guanylate cyclase.

It is now generally accepted that NO production is enhanced at all stages of cerebral ischemia [14]. The significant elevation of NO is most probably due to the induction of NOS by the ischemic condition. Hypoxia and hypercapnia also play important role in inducing NOS.

There is indirect evidence in human that high altitude hypoxic adaptation increase NO level in blood [27]. And in human cell culture, hypercapnia had been shown to produce increased NO [28]. These studies indicated that in patients who suffer from ischemic stroke there is a strong induction of NO production within the CNS, which is hypothesized to contribute to oxidative stress and subsequent neuronal damage.

Correlation between serum NO levels and Glasgow Coma Scale (GCS) score in ischemic stroke patients showed that serum NO levels in cases were negatively correlated with GCS score but the difference was statistically insignificant. Ozkul et al. in a study on 70 patients and 70 controls reported a significant elevation in serum NO levels in acute ischemic stroke as compared to controls. They also compared this elevated NO level with Canadian Neurological Scale (CNS) scores, which were negatively correlated; indicating NO has deleterious effects on outcome of ischemic stroke [29]. In contrast, study by Rashid et al. on 228 acute ischemic patient and 49 hemorrhagic stroke patient showed, plasma nitric oxide (NO), assessed as

nitrate/nitrite (NOx) level were low as compared to age and gender-matched controlled. And assessment of stroke severity with GCS score was positively correlated with plasma NO level, indicating NO may have some beneficial effect in acute ischemic stroke patient [21].

Therefore, NO released during acute ischemic stroke, may have some positive (neuroprotective) or, negative (neurodestructive) effect depending on the stage of the ischemic process and the isoform of NOS, which was upregulated during acute ischemic stroke. eNOS probably has some neuroprotective actions due to its effect on cerebral vasodilation as well as by inhibition of platelet aggregation [9]. In support of this, eNOS knockout mouse models of stroke developed larger cerebral infarct than the wild-type strain 24 h after middle cerebral artery (MCA) occlusion [30]. On the other hand, nNOS and iNOS have some neurodestructive effect [9]. Decrease cerebral infarct size were observed in mutant mice deficient in either neuronal NOS activity [31] or, iNOS gene activity [32] after MCA occlusion.

Conclusions

Significant elevation in mean serum NO levels and a low GCS score was observed in subjects of acute ischemic stroke. Serum NO levels in subjects of acute ischemic stroke were negatively correlated with GCS score but the difference was statistically insignificant. Findings in the present study suggest that the oxidative stress in acute ischemic stroke may be the result of imbalance in oxidant/antioxidant homeostasis. Further studies need to be carried out involving a much larger group of patients to establish this.

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Compliance with Ethical Standards

Conflict of interest There are no conflicts of interest.

References

- Johnson NB, Hayes LD, Brown K, Hoo EC, Ethier KA, Centers for Disease Control and prevention (CDC). CDC National Health Report: leading causes of morbidity and mortality and associated behavioral risk and protective factors—United States, 2005–2013. *MMWR Suppl* 2014;63:3–27.
- Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. *Circulation*. 2015;131:e29–322.
- Dalal PM, Malik S, Bhattacharjee M, Trivedi ND, Vairale J, Bhat P, et al. Population-based stroke survey in Mumbai, India: incidence and 28-day case fatality. *Neuroepidemiology*. 2008;31:254–61.
- Das SK, Banerjee TK, Biswas A, Roy T, Raut DK, Mukherjee CS, et al. A prospective community-based study of stroke in Kolkata, India. *Stroke*. 2007;38:906–10.
- Rothwell PM, Coull AJ, Giles MF, Howard SC, Silver LE, Bull LM, et al. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). *Lancet*. 2004;363:1925–33.
- Willmot M, Gibson C, Gray L, Murphy S, Bath P. Nitric oxide synthase inhibitors in experimental ischemic stroke and their effects on infarct size and cerebral blood flow: a systematic review. *Free Radic Biol Med*. 2005;39:412–25.
- Guix FX, Uribealago I, Coma M, Munoz FJ. The physiology and pathophysiology of nitric oxide in the brain. *Prog Neurobiol*. 2005;76:126–52.
- Szabo C. Physiological and pathophysiological roles of nitric oxide in the central nervous system. *Brain Res Bull*. 1996;41:131–41.
- Iadecola C. Bright and dark sides of nitric oxide in ischemic brain injury. *Trends Neurosci*. 1997;20:132–9.
- O'Mahony D, Kendall MJ. Nitric oxide in acute ischaemic stroke: a target for neuroprotection. *J Neurol Neurosurg Psychiatry*. 1999;67:1–3.
- Terpolilli NA, Moskowitz MA, Plesnila N. Nitric oxide: considerations for the treatment of ischemic stroke. *J Cereb Blood Flow Metab*. 2012;32:1332–46.
- 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;13:355–8.
- Kumura E, Kosaka H, Shiga T, Yoshimine T, Hayakawa T. Elevation of plasma nitric oxide end products during focal cerebral ischemia and reperfusion in the rat. *J Cereb Blood Flow Metab*. 1994;14:487–91.
- Kader A, Frazzini VI, Solomon RA, Trifiletti RR. Nitric oxide production during focal cerebral ischemia in rats. *Stroke*. 1993;24:1709–16.
- Castillo J, Rama R, Davalos A. Nitric oxide-related brain damage in acute ischemic stroke. *Stroke*. 2000;31:852–7.
- El Kossi MM, Zakhary MM. Oxidative stress in the context of acute cerebrovascular stroke. *Stroke*. 2000;31:1889–92.
- Aygun R, Kotan D, Demirbas F, Ulvi H, Deniz O. Plasma oxidants and antioxidants in acute ischaemic stroke. *J Int Med Res*. 2006;34:413–8.
- Xu L, Wang B, Kaur K, Kho MF, Cooke JP, Giffard RG. NOx and ADMA changes with focal ischemia, amelioration with the chaperonin GroEL. *Neurosci Lett*. 2007;418:201–4.
- 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;734:86–90.
- Ferlito S, Gallina M, Pitari GM, Bianchi A. Nitric oxide plasma levels in patients with chronic and acute cerebrovascular disorders. *Panminerva Med*. 1998;40:51–4.
- 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;12:82–7.
- 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;10:473–6.
- Beridze M, Momtselidze N, Shakarishvili R, McHedlishvili G. Effect of nitric oxide initial blood levels on erythrocyte aggregability during 12 hours from ischemic stroke onset. *Clin Hemorheol Microcirc*. 2004;30:403–6.

24. Woodruff TM, Thundiyil J, Tang SC, Sobey CG, Taylor SM, Arumugam TV. Pathophysiology, treatment, and animal and cellular models of human ischemic stroke. *Mol Neurodegener.* 2011;6:11.
25. Deb P, Sharma S, Hassan KM. Pathophysiologic mechanisms of acute ischemic stroke: an overview with emphasis on therapeutic significance beyond thrombolysis. *Pathophysiology.* 2010;17:197–218.
26. Xing C, Arai K, Lo EH, Hommel M. Pathophysiologic cascades in ischemic stroke. *Int J Stroke.* 2012;7:378–85.
27. Beall CM, Laskowski D, Erzurum SC. Nitric oxide in adaptation to altitude. *Free Radic Biol Med.* 2012;52:1123–34.
28. Fathi AR, Yang C, Bakhtian KD, Qi M, Lonser RR, Pluta RM. Carbon dioxide influence on nitric oxide production in endothelial cells and astrocytes: cellular mechanisms. *Brain Res.* 2011;1386:50–7.
29. Ozkul A, Akyol A, Yenisey C, Arpaci E, Kiylioglu N, Tataroglu C. Oxidative stress in acute ischemic stroke. *J Clin Neurosci.* 2007;14:1062–6.
30. Huang Z, Huang PL, Ma J, Meng W, Ayata C, Fishman MC, et al. Enlarged infarcts in endothelial nitric oxide synthase knockout mice are attenuated by nitro-L-arginine. *J Cereb Blood Flow Metab.* 1996;16:981–7.
31. Huang Z, Huang PL, Panahian N, Dalkara T, Fishman MC, Moskowitz MA. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science.* 1994;265:1883–5.
32. 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;17:9157–64.