Incidence and Clinical Outcome of Patients with Hypertensive Acute Ischemic Stroke: An Update from Tertiary Care Center of Central India

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ABSTRACT

Introduction: We evaluated the incidence and clinical outcome of patients with hypertensive acute ischemic stroke (AIS) admitted to a tertiary care center in Central India. In addition, we examined the status of stroke biomarkers namely neuron-specific enolase (NSE), glial specific protein (S-100ββ), and inter-α-trypsin inhibitor heavy chain 4(ITIH4) in the serum of patients suffering from AIS with hypertension (HTN) and without HTN.

Methods: A total of 104 patients with AIS were enrolled for the study. Clinical outcome and stroke biomarker levels were evaluated in them at the time of hospital discharge and then followed at 12 months and 18 months after hospital discharge.

Results: HTN is a major risk factor associated with 67%(70.104) of patients with AIS. Multivariate analysis suggests higher odds of 4.088(95%Cl, 0.721–23.179) and 2.437(95%Cl, 0.721–23.179) for 12 and 18 months outcome in patients with AIS and HTN, respectively. Serum NSE and S-100ββ decreased at the time of discharge as compared to admission level in improved patients suffering from AIS with or without HTN, whereas levels of ITIH4 peptides 2 and 7 increased at the time of discharge (compared to its admission level) only in improved patients with AIS regardless of HTN or non-HTN condition.

Conclusion: HTN is one of the major risk factors associated with higher risk of AIS as well as long-term unfavourable outcome after AIS in Central India region. NSE, S-100ββ, and ITIH4 were found to be independent predictors of outcome in patients with AIS irrespective of HTN and non-HTN condition.

1. Introduction

Stroke is still the third leading cause of death worldwide (Dichgans, 2007). According to the 2002 survey, South Asian countries such as Sri Lanka, India, Pakistan, and Bangladesh account for 20% of 5.54 million deaths caused by stroke worldwide. Several modifiable and non-modifiable factors such as hypertension (HTN), smoking, diabetes mellitus (DM), hyperlipidemia, obesity, sedentary

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lifestyle, cardiac abnormalities (e.g. arterial fibrillation), age, and sex have been reported to increase the risk of acute ischemic stroke (AIS) (O'Donnell et al., 2010; Engelter, Bonati, & Lyrer, 2006; Reynolds et al., 2003; Lawes, Vander Hoorn, & Rodgers, 2001). Amongst the reported risk factors, HTN is the most important risk factor, which has been in constant debate since the mid-20th century for its effects (Kearney, Whelton, Reynolds, Whelton & He, 2004).

Severe HTN in the early phase of stroke is associated with poor prognosis and high mortality rates in patients with AIS (Chamorro et al., 1998). HTN is known to contribute for more than 57% of deaths in stroke patients (Gupta, 2004). Earlier epidemiological studies on HTN in Indian population revealed that incidence of HTN was increasing in India (Anchala et al., 2014). A recent study on HTN has also anticipated that current increase in HTN cases in India will probably result in large number of HTN-stroke patients and has recommended to start a HTN-stroke control program in India (Dalal & Bhattacharjee, 2007).

In spite of that, there is a paucity of recent data on HTN-stroke in Indian population. In the present study, we aimed to evaluate HTN in patients with AIS admitted in a tertiary health care center of Central India and studied its association with prognosis of AIS. In addition, we also compared the status of stroke biomarkers namely neuron-specific enolase (NSE), glial specific protein (S-100ββ), and inter-α-trypsin inhibitor heavy chain 4 (ITIH4) in the serum of these patients with and without HTN.

2. Methods

2.1. Ethical statement

The study was approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences (CIIMS), Nagpur.

2.2. Patients

The study was carried out in 104 selected patients with AIS admitted between October 2011 to December 2013 in CIIMS, Nagpur after taking their informed consents and kin of the patients for the study. The patients were admitted to the Intensive Care Unit (ICU) where the ambient temperature was maintained between 20°C and 25°C. Diagnosis of AIS was done on the basis of WHO definition of stroke, i.e. rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin.

Detailed history and CT scans within 12 hours of admission were taken to rule out stroke mimic and other conditions such as hemorrhagic stroke, brain malignancies, and transient ischemic attack. Patients who underwent brain operation or were presented with severe systemic disease, dementia, psychiatric disease, active infection, were excluded from the study. Similarly, patients who took discharge against medical advice, were also excluded from the study.

A predesigned proforma-based questionnaire was recorded and maintained for all patients with AIS. A baseline of clinical characteristics such as age, gender, risk factors, behavioral factors, past history of stroke, and duration of hospital stay were also recorded. Smoking was defined as current use of >1 cigarette per day. Diabetes mellitus (DM) was defined as receiving oral hypoglycemic agents/insulin treatment with glycosylated hemoglobin. HTN in patients with AIS was defined as having blood pressure ≥140/90 mm Hg on repeated measurements during the hospitalization or when the patient was on anti-hypertensive medication.

Stroke severity was evaluated using National Institute of Health Stroke Scale (NIHSS). The NIHSS score consists of 15 items and a total score of 42 points. Scores of 0=no stroke, 1–6=minor stroke, 7–18=moderate stroke, and 19–42=severe stroke. The modified Rankin Scale (mRS) was used for evaluation of outcome in patients with AIS. Based on mRS score, the patients were classified into 2 outcome groups; improved group (n=87; mRS score 0-2); and dependent /expired group (n=17; mRS score 3-6).

To study the impact of HTN on a long-term functional outcome, follow-up study was carried out at 12 and 18 months after discharge. Participants were recruited based on earlier records available at the hospital. Telephone follow-up was performed consisting of a short questionnaire from participants or their kin, about the level of improvement/dependency or death after discharge.

2.3. Samples

Blood samples were collected from all patients with AIS at the time of admission and then at the time of discharge or expiry. Serum was separated and stored at -20°C until further use.

2.4. Designing and synthesis of peptides and anti-peptides of inter-α-trypsin inhibitor heavy chain 4

The reference sequence of inter-α-trypsin inhibitor heavy chain 4 (ITIH4) was obtained from NCBI (National Center for Biotechnology Information) reference sequence data-

bases. The antigenic peptides of ITIH4 were determined on the basis of Kolaskar and Tongaonkar (1990) method using online software, Molecular Immunology Foundation-Bioinformatics (MIF-Bioinformatics software) (Kolaskar & Tongaonkar, 1990). These antigenic peptide sequences were then subjected to multiple sequence alignment using NCBI BLAST (Basic Local Alignment Search Tool) to obtain the sequence similarities with other non-redundant protein database sequences. Based on the results of the blast analysis, 9 antigenic sequences of ITIH4 were selected. These peptide sequences were then sent for antibody production to GenicBio Lab, Shanghai, China (Table 1).

2.5. Estimation of inter-α-trypsin inhibitor heavy chain 4 using anti-peptide antibody

An in-house ELISA method was employed for detection of ITIH4 in patients with AIS. Briefly, 96 well micro-titer plates were coated with 100µL serum samples and incubated for 45 minutes at 37ºC. The plates were washed once with wash buffer, i.e. 0.5% tween-20 in phosphate buffered saline (PBST) and then blocked by addition of 200µL of blocking buffer (0.5% BSA in PBST). After 90 minutes of incubation, 100 µL of ITIH4 anti-peptide antibody (GenicBio Lab, Shanghai, China) was added and further incubated for 45 minutes. For color development, 100 µL of 1:10000 dilution of anti-rabbit Horseradish peroxidase (HRP) conjugated secondary antibody (Merck Millipore, India) was added and incubated at 37°C for 45 minutes. The reaction was stopped with 100 µL H2SO4(2.5 N) and intensity of color developed was measured at 450 nm using an ELISA reader.

2.6. Neuron-specific enolase estimation

Serum levels of neuron-specific enolase (NSE) were estimated with CanAg NSE EIA kit as per the instructions of manufacturer (Fujirebio Diagnostics AB, Sweden). The assay is based on solid phase, non-competitive immunoassay based on 2 monoclonal antibodies (derived from mice) directed against 2 separate antigenic determinants of NSE molecule. The monoclonal antibodies (MAb) were used to bind to γ subunit of enzyme and thereby detect both γγ and αγ isoenzymes of NSE. In brief, the method is to transfer the required number of microplate strips to a strip frame. Each strip was washed once with wash solution. About 25 µL of the NSE calibrators (CAL A, B, C, D, E) along with patient's specimens (unknowns-Uk) was pipetted into the strip wells. The plate was incubated for 1 hour (±10 min) at room temperature (20°C-25°C) with constant shaking of the plate using a microplate shaker. After incubation, each strip was aspirated and washed 6 times. Then, 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) HRP-substrate was added to each well and incubated for 30 minutes (±5 min) at room temperature with constant shaking. After incubation, 100 µL of stop solution was added, mixed and the absorbance was read at 405 nm in a microplate spectrophotometer within 15 minutes after addition of stop solution.

2.7. Glial specific protein S-100ββ estimation

In vitro assay for the quantitative determination of S-100ββ (glial specific protein) in human serum was performed as per the instructions indicated in the manual CanAg S-100 EIA (Fujirebio Diagnostics AB, Sweden). This is a 2-step enzyme immunometric assay based on 2 monoclonal antibodies derived from mouse specific for 2 different epitopes of S-100ββ. Thus, the assay determines both S-100αβ and S-100ββ without cross-reactivity with other forms of S-100.

In brief, ELISA wells were washed once with wash solution. Then, 50 µL of S-100ββ calibrators and samples

Table 1. List of anti-peptide antibody generated against the selected peptides of ITIH4.

Antibody	Sequence
Anti ITIH-4 Peptide-1	LLLKVRPQQLVKH-C
Anti ITIH-4 Peptide-2	REALIKILDD-C
Anti ITIH-4 Peptide-3	PEGSVSLIILLT-C
Anti ITIH-4 Peptide-4	RYSLFCLGFGFDVSY-C
Anti ITIH-4 Peptide-5	C-GPDVLTATVSGK
Anti ITIH-4 Peptide-6	C-LNLSLAYSFV
Anti ITIH-4 Peptide-7	C-TFFKYYLQGAKIPKPEA
Anti ITIH-4 Peptide-8	C-LLLSDPDKVT
Anti ITIH-4 Peptide-9	C-LGQFYQEVLWG

were pipetted into the strip wells. About 100 µL Biotin anti-S-100ββ was added to each well and the frame was incubated containing the strips for 2 hours (±10 min) at room temperature (20°C–25°C) with constant shaking of the plate using a microplate shaker. After incubation, each strip was aspirated and washed 3 times using the wash buffer. Next, 100 µL of Tracer working solution was added to each well. The frame was incubated for 1 hour (±5 min) at room temperature with constant shaking. After incubation, each strip was aspirated and washed 6 times. Afterwards, 100 µL of TMB HRP-Substrate was added to each well and incubated for 30 minutes (±5 min) at room temperature with constant shaking. Then, 100 µL of stop solution was added and mixed. Absorbance was recorded at 405 nm in a microplate spectrophotometer within 15 minutes after addition of stop solution.

2.8. Statistical analysis

Demographic and baseline characteristics among patients suffering from AIS with HTN or without HTN were analyzed using the Chi-square test. P<0.5 was considered statistically significant. Adjusted odds ratio for analysis of long-term outcome was studied using multivariate analysis by adjusting confounding factors such as age, sex, diabetes, smoking, alcohol, NIHSS score, and admission time. Kaplan-Meier analysis was performed to determine the long-term survival in HTN and non-HTN patients with AIS. All these analyses were performed using R-programming language (version: 3.0.0.0)

3. Results

Out of 104 patients with AIS, 70(67%) had HTN and 34(23%) did not have HTN. The baseline characteristics of HTN and non-HTN patients with AIS are tabulated in Table 2. We observed that HTN was more frequent (76%) among the old (>50 years) patients with AIS. In spite of insignificant P-value (0.097), HTN females were found to be more prone to AIS (36%) as compared to non-HTN females (18%). DM was found to be more frequent (P<0.05) in patients with HTN (37%) as compared to non-HTN (9%) patients. It was also observed that systolic pressure (152.09±25.85 mm Hg) was higher

Table 2. Clinical characteristics of patients with AIS with and without HTN.

Characteristics		No.	Hypertension (n=70)	Without hypertension (n=34)	P-value
Age	≤50 years	38	17(24%)	21(62%)	0.0005
	>50 years	66	53(76%)	13(38%)	
Sex	Male	73	45(64%)	28(82)	0.097
	Female	31	25(36%)	6(18%)	
Admission within 24h		48	33(47%)	15(44%)	0.936
Diabetes		29	26(37%)	3(9%)	0.005
Ischemic heart diseases		05	5(7%)	0	0.2676
Cardiac disease		03	3(4%)	0	0.548
History of smoking		08	4(6%)	4(12%)	0.488
History of alcohol		11	8(11%)	3(9%)	0.948
Past history of stroke		06	5(7%)	1(3%)	0.679
Pulse rate		104	79.439±13.322	78.759±11.984	0.806
BP	Systolic	104	152.09±25.85	135±20.945	0.0007*
	Diastolic	104	90.537±14.29	85.125±15.837	0.107
Thrombolysis		10	8(11%)	2(6%)	0.585
Decompression surgery		03	1(1%)	2(6%)	0.517
Disability score on admission†	Severe	14	9(13%)	5(15%)	0.134
	Moderate	44	28(40%)	16(47%)	
	Mild	23	20(28%)	3(9%)	

*P-value<0.05 is considered significant.

Table 3. Comparison of outcomes in patients with AIS with and without HTN.

Characteristics	n	Hypertension	Without hypertension	P-value
Hospital stay		**n=70**	**n=34**	
≤15 Days	87	60(86%)	27(79%)	0.594
>15 Days	17	10(14%)	7(21%)	
Final outcome				
Dependent/expired	17	12(17%)	5(15%)	0.974
12 month outcome†		**n=42**	**n=18**	
Dependent/expired	20	18(43%)	2(11%)	0.136
18 month outcome†		**n=38**	**n=18**	
Dependent/expired	16	14(37%)	2(11%)	0.218

†out of 60.

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Table 4. Unadjusted and adjusted odds ratios of clinical outcome in patients with AIS with HTN and without HTN.

Outcome	Unadjusted OR (95%CI)	†Adjusted OR (95%CI)
Outcome at discharge		
Dependent/expired (n=17)	1.18(0.39-4.10)	6.00(0.715-50.375)
Long-term outcome		
At 12 months		
Dependent/expired (n=20)	5.53(1.31-41.8)	4.088(0.721-23.179)
At 18 months		
Dependent/expired (n=16)	4.32(1.00-33.11)	2.437(0.395-15.05)

OR: indicates Odds Ratio, CI, Confidence Interval.

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†Adjusted for age, sex, diabetes, smoking, alcoholic habit, disability score (NIHSS score), and admission time.

(P<0.0007) in HTN-AIS patients as compared to non-HTN AIS patients (135±20.945 mm Hg).

Table 3 shows the comparison of outcomes in our patients with and without HTN. Results of death or dependency in patients with AIS at the time of hospital discharge were similar between HTN (17%, 12.70) and non-HTN (15%, 5.34) patients with AIS. Long-term outcome at 12 months and 18 months showed higher ratio of dependency/expired outcome in HTN-AIS patients (43%, 18.42 and 11%, 2.18) as compared to non-HTN patients with AIS (37%, 14.38 and 11%, 2.18), respectively.

A survival curve (log-rank analyses) for the mortality and morbidity in the evaluated patients was prepared to study the impact of HTN on the survival of patients with AIS. Figure 1 shows a higher (Log-rank P=0.042) mortality and morbidity in hypertensive patients with AIS (green line) as compared to normotensive patients with AIS (blue line).

Univariate logistic regression analysis suggests that patients with AIS and HTN were at higher risk of death and dependency at discharge (odds ratio: 1.18, 95%Cl, 0.39–4.10), at 12 months (odd ratio: 5.53, 95%Cl, 1.31–41.8) and at 18 months of follow-up (odd ratio: 4.32, 95%Cl, 1.00–33.11) even after adjusting for potential confounders in multivariate logistic regression (Table 4). HTN was found to be an independent risk factor for death and dependency at discharge (odd ratio: 6.00; 95%Cl, 0.715–50.375) at 12 months of follow-up (odd ratio: 4.088; 95%Cl, 0.721–23.179) and at 18 months of follow-up (odd ratio: 2.437, 95%Cl, 0.395–15.05) after the onset of AIS.

The level of ITIH4 (peptide 2 and 7), NSE, and S100ββ in patients suffering from AIS with HTN and without HTN are shown in Figures 2 and 3. Results showed that the levels of ITIH4 peptide 2 and 7 increased at the time of discharge (compared to its admission level) in improved patients with AIS regardless of their HTN or non-HTN condition. However, the levels of same peptides decreased (P=0.05 for Peptide 7) at the time of discharge in expired/dependent patients with AIS (Figure 2).

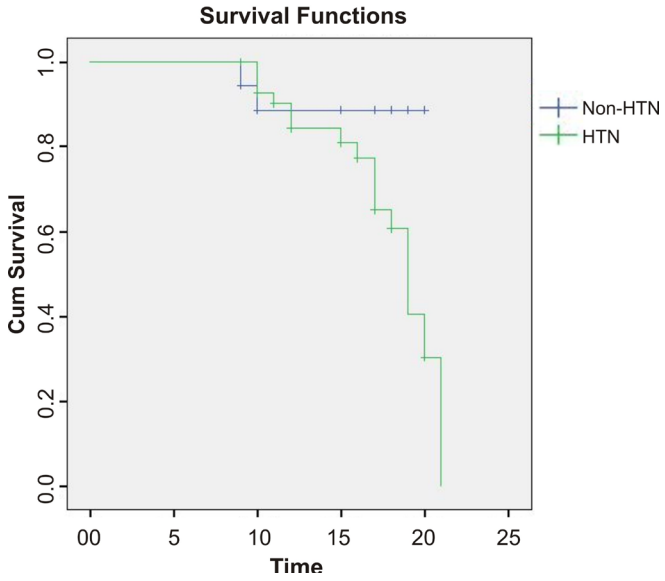

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Figure 1. The Kaplan-Meier survival curves for HTN (green line) and non-HTN (blue line) patients with AIS based on 18 month mRS score.

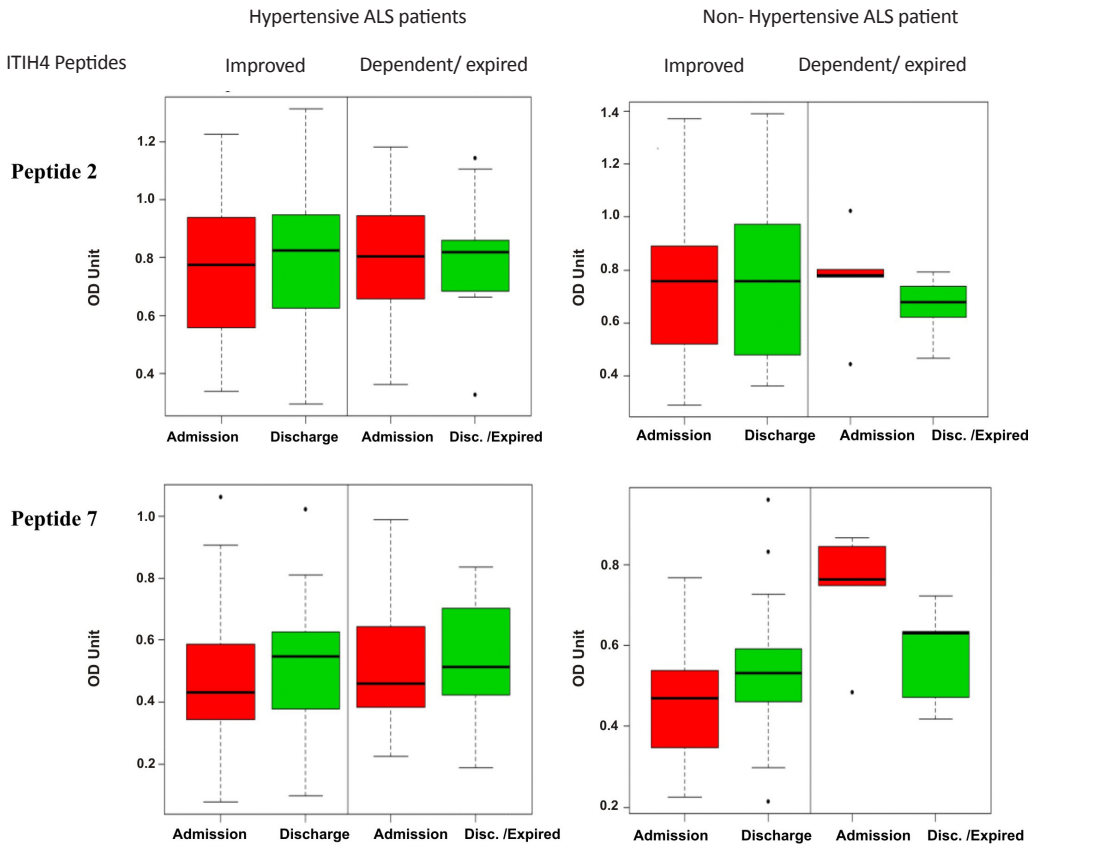

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Figure 2. Level of ITIH4 in HTN (n=70) and non-HTN (n=34) patients with AIS stroke with improved and dependent/expired outcome.

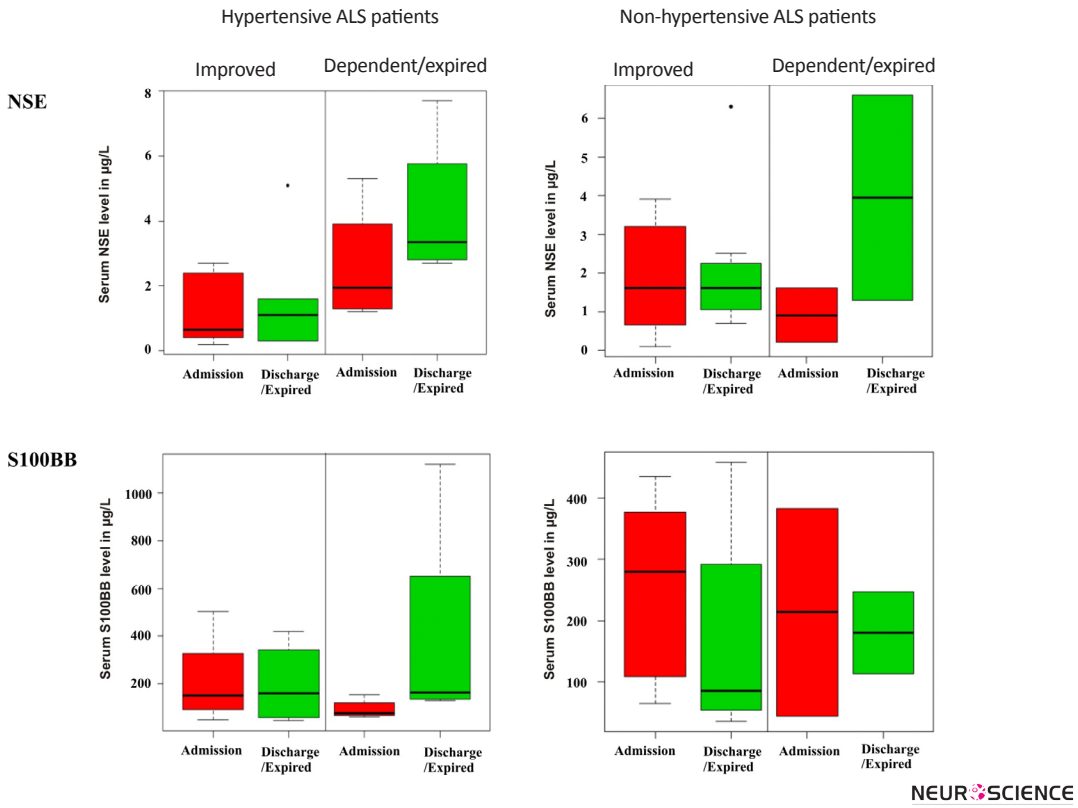

Figure 3. Level of NSE and S-100ββ in HTN (n=70) and non-HTN (n=34) patients with AIS stroke with improved and dependent/expired outcome.

The levels of NSE and S100ββ decreased at the time of discharge (as compared to admission levels) in improved HTN and non-HTN patients with AIS. However, the levels of same markers were found high in HTN and non-HTN patients with AIS with dependent/expired outcome in the hospital (Figure 3).

4. Discussion

In the present study, we evaluated HTN in patients with AIS admitted to a tertiary health care center of Central India and studied its association with the outcome of AIS. We observed that HTN is an associated risk factor in 67% of total AIS cases and is more frequent (76%) among the older (>50 years) patients. Although HTN did not affect hospital outcome significantly, it was associated with post discharge (12 months and 18 months) dependency/expired outcome in patients with AIS. After adjusting potential confounders, multivariate analysis suggests that HTN increases the risk of dependency/expired outcome in patients with AIS during the postdischarge period (12 months and 18 months).

HTN is one of the important risk factors impacting the majority of the patients with AIS (Shah et al., 2013). It increases the risk for both ischemic and hemorrhagic stroke, both in patients with history of coronary heart disease and stroke (Gaciong, Siński, & Lewandowski, 2013). Similarly, a significant association has been reported between HTN and stroke recurrence in patients with AIS and small-vessel diseases (Wang et al., 2013). Recent report in India suggests that HTN exists in 12% to 40% (Taylor & Suresh, 2012) of patients with AIS. However, we observed high incidence of HTN (67%) in the patients with AIS, indicating more complicated scenario in Central India compared to reports from rest of the India (Anchala et al., 2014).

HTN apart from being a risk factor for the occurrence of AIS, has also been implicated to be associated with an unfavorable outcome in patients with AIS. Zhang et al. reported a significantly positive association of high systolic and diastolic blood pressure with death and disability among patients with hemorrhagic stroke but not with AIS (Zhang et al., 2008).

On the other hand, Brush et al. reported that HTN would increase the risk of mortality by 4.5 fold among childhood patients with AIS during the postdischarge period of 12-months (Brush, Monagle, Mackay & Gordon, 2013) which is in the agreement with our current study results. We found higher odds of 4.088 and 2.437 for

dependency/expired outcome in hypertensive AIS patients at postdischarge period of 12 and 18 months, respectively. This finding suggests that acute management of HTN is of utmost importance for reducing the risk of unfavorable outcomes in patients with AIS during their rehabilitation after hospital discharge. The study also advocates that similar analysis should also be conducted in other parts of the country.

We further investigated the impact of HTN on the stroke biomarkers namely NSE, S-100ββ, and ITIH4 (Peptide 2 and 7) in the serum of patients with AIS. NSE is a dimeric isoenzyme of the glycolytic pathway of neuronal cells in brain (Brea et al., 2009). Serum NSE level has been reported to be a useful biomarker for early prediction of severity and outcome in patients with AIS (Zaheer et al., 2013). Similarly, S100ββ is a $Ca^{2\pm}$ binding protein found in glial cells in the central nervous system. Increased S-100ββ is also a well-known marker for brain damage in stroke patients (Selakovic, Raicevic, & Radenovic, 2005; Wunderlich, Lins, Skalej, Wallesch, & Goertler, 2006) which correlates with its severity and outcome (Brouns et al., 2010).

In our earlier studies, we have reported that (ITIH4) which is a 120-kDa serum protein, normally presents in the serum of healthy subjects but very low or absent in patients with AIS (Nayak et al., 2012). We also compared S-100ββ and NSE protein levels across the same time intervals and found that serum level of ITIH4 correlated with S-100ββ and NSE concentrations. Thus, ITIH4 could be a useful biomarker for prognosis of patients with AIS (Nayak et al., 2012; Kashyap et al., 2009). In the current study, we found that the levels of ITIH4 peptide 2 and 7 increased at the time of discharge as compared to its admission value in improved patients with AIS as compared to expired/dependent patients. The changes observed in NSE, S-100ββ, and ITIH4 are similar in both HTN and non-HTN patients. This suggests that NSE, S-100ββ, and ITIH4 are independent predictors of outcomes in patients with AIS and remain unaffected by HTN.

Despite the fact that this is the first study in Central India population showing the association of HTN with clinical outcome of patients with AIS and suggesting the independent role of ITIH4, NSE, and S100ββ in stroke, it suffers from some limitations. First, the study was limited by small samples size to be represented as Central Indian population. Also despite our extensive efforts, follow-up data of only few patients (i.e. clinical status at 12 and 18 months after hospital discharge) were available during the study. Nevertheless, the obtained findings were encouraging and has set a platform for further evaluation in large scale study.

In conclusion, results of the present study suggest that HTN is the major risk factor for AIS, as well as unfavourable outcomes after AIS, in Central India region. Also, HTN does not influence the performance of stroke biomarkers NSE, S100ββ, and ITIH4, suggesting their independent role in patients with AIS.

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Conflict of Interest

All authors declared no conflict of interest.

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