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Original Article

Biochemistry Section

Impact of Admission Time on Treatment and Outcome of Stroke in Patients Admitted to Tertiary Care Hospital: A Pilot Study from Central India

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ABSTRACT

Introduction: Admission of patients within window period has been linked with efficacy of treatment outcome and recovery. The present study examined the effects of early vs delayed admission on functional outcome of Acute Ischemic Stroke (AIS) as well as added value of stroke markers in such patients admitted to a tertiary care hospital in Central India.

Materials and Methods: Hundred and four patients admitted to Neurology department of Central India Institute of Medical Sciences were grouped as early referrals (within 24 hour admission) and late referrals (after 24 hour admission) based on onset of symptoms and time of admission. Baseline data, throm bolysis eligibility, hospital and long term outcomes were determined in early and later referrals. Stroke markers NSE, S-100 ββ and ITIH4 peptides were also screened in patients who were further categorized as improved and expired /dependent during hospital outcome. Outcome of death /dependency in both groups was analysed using multivariate regression analysis. Kaplan-Meier analysis was performed to determine the rate of stroke-mortality in hospital and over 12 and 15 month period.

Results: Hospital outcome indicated higher percentage (90%) of improved cases in early referrals as opposed to 79% observed in late referrals. Similarly, the ratio of dependency was slighter higher in late referrals (18%) as compared to early referral (6%) cases. The long term outcome at 12 and 18 months showed more or less similar ratio of death/dependency in early (23%, 9%) and late referrals (32%,24%) respectively. Multivariate analysis revealed no significant impact of risk confounders at long term and short term outcome in both groups. Analysis of stroke marker revealed better prognosis with significant association between ITIH4 peptides and NSE & S-100 ββ level with level of improvement in early referrals.

Conclusion: Early admission of AIS patients is associated with better hospital outcome. However admission time has no major impact on long term outcome in AIS patients. Moreover, stroke markers such ITIH4, can be used as a predictor of stroke outcome and may have prognostic importance in AIS cases in future.

Keywords: Biomarkers, ITIH4 protein, Neuron-specific enolase, Prognosis, Stroke

INTRODUCTION

Stroke remains one of the leading causes of mortality in India [1]. The estimated prevalence rates of stroke ranges from, 84-262/100,000 in rural and 334-424/ 100,000 in urban areas [2]. Acute Ischemic Stroke (AIS) is a clinical emergency that requires immediate and prompt treatment, with bench-marked timelines mandated for every step in the process from admission to the point of discharge. As per the guidelines of the National Institute of Neurological Disorders and Stroke (NINDS), transferring a stroke patient to an inpatient setting should be achieved within 3 hours of arrival [3]. The use of alteplase for thrombolysis within the first 4.5 hours after stroke onset is the only approved pharmaceutical treatment for AIS, with significantly improved clinical outcomes. Window period from point of occurrence of stroke to admission of patients in emergency wards thus constitutes an important part of treatment to be given and is associated with better recovery rates.

Animal studies on neurobiological mechanism of post-stroke recovery have demonstrated that there is a period of time shortly after a stroke event when the brain appears to be primed for recovery by a series of neuroreparative events [4]. Similar to studies in animals, investigators have demonstrated that delays between the onset of stroke and the commencement of therapeutic rehabilitation in humans have been associated with poor outcomes [5]. Over the past few years, analysis of brain damage markers have attracted considerable attention in variety of central nervous system (CNS) disorders. Neuron-specific enolase (NSE) and S100 ββ protein are implicated in several brain injuries, including stroke [6]. High levels of NSE and S100 ββ have been linked to poor outcomes in AIS patients [7]. In our earlier studies we have identified and reported inter-α-trypsin inhibitor heavy chain 4 (ITIH4) proteins with low expression levels in serum of AIS patients [8]. Based on identified markers, our laboratory has developed synthetic peptide based assay for early diagnosis and prognosis in AIS patients. Utility of such markers in serum of early and late admission AIS cases therefore may be valuable with respect to diagnosis and prognosis and determining severity of outcome in early vs delayed AIS admitted patients.

So far there are only limited studies in India that describe relevance of post stroke admission time on treatment outcome and recovery in AIS patients. Moreover, no studies have been carried out on utility of NSE, S-100 ββ, and ITIH4 peptide based assay and its correlation with early vs delayed admission in Central India. The present study therefore was carried out with an aim to study effects of early vs delayed admission on functional outcome as well as to study the added value of stroke biomarkers in AIS patients admitted to tertiary care hospital in Central India.

MATERIALS AND METHODS

Study Subjects

The present study was undertaken among 104 subjects admitted to IPD wards and intensive care unit (ICU) of Central India Institute of Medical Sciences (CIIMS) Nagpur, India from November 2012 to January 2014. The Diagnosis of AIS was done in accordance with WHO guidelines for stroke which includes “Rapidly developing signs of focal (or global) disturbance of cerebral function lasting >24 hours (unless interrupted by surgery or death), with no apparent non

vascular cause, history, neurological examination and Computerized tomography (CT)". Baseline data such as age, sex, along with detailed history of risk factors linked to AIS such as hypertension, ischemic heart disease (IHD), smoking, alcohol consumption, any past history of stroke were recorded. Exclusion of subjects mimicking similar outcomes to that of stroke was done based on CT scan analysis. Apart from that, patients in older age groups (85 years or more); haemorrhagic stroke patients; brain malignancies patients; transient ischemic attack; patients who underwent brain operation; patients who presented with severe systemic disease, dementia, psychiatric disease, active infection, refused and patients who took discharge against medical advice were excluded from the study

Subjects were classified based on time period of admission in Neurology and Emergency wards post events of the stroke. Based on the following admission criteria, the study subjects were classified into two different groups, those admitted within 24 hours (early referrals) after occurrence of stroke event and those admitted after 24 hours post stroke event (late referrals). Details of any thrombolysis treatment given, and decompression surgery done in patients in both groups were also recorded. Severity of stroke on admission was evaluated using National Institute of Health Stroke Scale (NIHSS). The modified Rankin Scale (mRS) was used for evaluation of outcome in AIS patients by a certified investigator at discharge. Based on mRS Score, AIS patients were classified into two outcome groups which includes improved group (n = 87; m-RS score 0-2); and dependent group (n = 17; m-RS score 3-6).

Follow Up

To study impact of admission time on long term functional outcome, follow up study was carried out at 12th and 18th months post hospital discharge. Participants were recruited based on earlier records available at hospitals. Telephonic follow up was taken, which consisted of short questionnaire from participants or their kin, about level of improvement/dependency or death post discharge. All follow up cases were further correlated with level of improvement/ dependency at time of hospital outcome to study prognostic outcome based on admission time. The detailed recruitment of participants during hospital and follow up including loss of cases in follow up is mentioned in [Table/Fig-1].

Samples

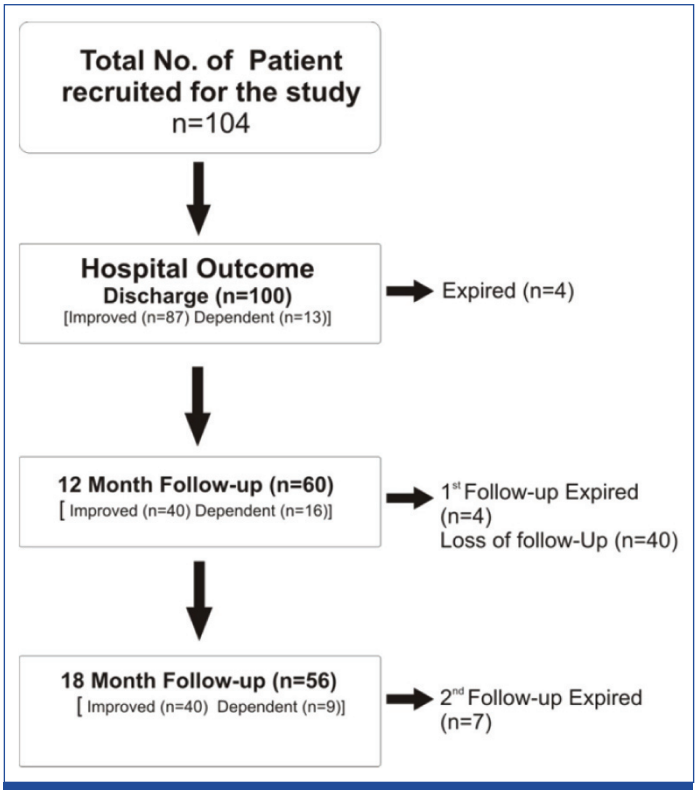
For evaluation of stroke markers: NSE, S-100 ββ and ITIH4 peptides, blood was collected from both patient groups post admission and at discharge/on death and serum was separated and stored at -20°C until further use.

Designing and Synthesis of Peptides and Anti-peptide of ITIH4

The reference sequences of ITIH4 were obtained through NCBI reference sequence databases. The antigenic peptides of ITIH4 were determined on the basis of Kolaskar and Tongaonkar (1990) method by using online software titled "Molecular Immunology Foundation-Bioinformatics software (MIF-Bioinformatics software)". These antigenic peptide sequences were then subjected to multiple sequence alignment using NCBI BLAST to obtain the sequence similarities with other non redundant protein database sequences. Based on the results of the blast analysis, two antigenic sequences of 9 sequences of ITHIH4 were selected. These peptide sequences were then sent for antibody production in GenicBio lab, Shanghai, China [Table/Fig-2].

Estimation of ITIH4 Using Anti-peptide Antibody

An in house ELISA method was employed for detection ITIH4 peptides in AIS patients. Briefly, 96 well microtiter plates were coated with 100µL serum samples and incubated for 45 minutes at 37°C. The plates were washed once with wash buffer, i.e. 0.5% Tween® 20 in phosphate buffered saline (PBST) and then blocked by



[Table/Fig-1]: Study flow diagram

S No.	Antibody produced	Start position	Antigenic sequence of ITIH4	End position
1	Anti ITIH4 Peptide -1	159	LLLKVRPQQLVKH-C	171
2	Anti ITIH4 Peptide -2	294	REALIKILDD-C	303
3	Anti ITIH4 Peptide -3	373	PEGSVSLIILLT-C	384
4	Anti ITIH4 Peptide -4	409	RYSLFCLGFGFDVSY-C	423
5	Anti ITIH4 Peptide -5	501	C-GPDVLTATVSGK	512
6	Anti ITIH4 Peptide -6	576	C-LNLSLAYSFV	585
7	Anti ITIH4 Peptide -7	623	C-TFFKYYLQGAKIPKPEA	639
8	Anti ITIH4 Peptide -8	835	C-LLLSDPDKVT	844
9	Anti ITIH4 Peptide -9	875	C-LGQFYQEVLWG	885

[Table/Fig-2]: List of anti ITIH4 peptide antibody produced against the selected antigenic sequence of ITIH4

addition of 200 µL of blocking buffer (0.5% BSA in PBST). After 90 minutes of incubation, 100µL of primary ITIH4 anti-peptide antibody rose in rabbit) was added and further incubated for 45 minutes. For colour development,100 µL anti-rabbit HOURP conjugated secondary antibody (dilution 1:10,000) was added and incubated at 37°C for 45 minutes. The reaction was stopped with 100µl H_2SO_4 (2.5 N) and intensity of colour developed was measured at 450 nm using an ELISA reader.

NSE Measurement

NSE serum levels were estimated with Can Ag NSE EIA (Sweden) as per the instructions of kit manufacturer. Test is based on solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of NSE molecule. The monoclonal antibodies (MAb) used bind to γ subunit of enzyme and thereby detect both γγ and αγ is enzymes of NSE. In brief the method is to transfer the required number of microplate strips to a strip frame. Wash each strip once with the wash solution. Pipette 25 µL of the NSE Calibrators (CAL A, B, C, D, E) and patient specimens (unknowns-Uk) into the strip wells. Incubate the plate for 1 hour (±10 minutes) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker. After the incubation

aspirate and wash each strip 6 times. Add 100 µL of TMB HOURP-Substrate to each well. Incubate for 30 minutes (± 5 minutes) at room temperature with constant shaking. After incubation add 100 µL of stop solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 minutes after addition of stop solution.

S-100 ββ measurement

IThe instructions, as in the manual (Can Ag S-100 EIA Sweden), was followed for the in vitro assay for the quantitative determination of S-100 ββ in human serum. The assay determines both S-100αβ and S-100ββ without cross reactivity with other forms of S-100 as it is a two-step enzyme immunometric assay based on two monoclonal antibodies derived from mouse specific for two different epitopes of S-100ββ.

In brief, the method is as follows : ELISA wells were washed once with wash Solution. 50 µL of S100ββ calibrators and sample into the strip wells are pipette. 100 µL Biotin Anti-S100ββ ββ was added to each well and the frame was incubated, containing the strips for two hours (± 10 minutes) at room temperature (20–25°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. 100 µL of tracer working solution was added to each well. The frame was incubated for one hour (±5 minutes) at room temperature with constant shaking. After incubation each strip was aspirated and washed 6 times. 100 µL of TMB/H_2O_2 substrate was added to each well. Incubation was done for 30 minutes (± 5 minutes) at room temperature with constant shaking. 100 µL of stop solution was added. At 405 nm it was mixed and absorbance read, in a microplate spectrophotometer within 15 minutes after addition of Stop solution.

Ethics

Informed consents were taken from all enrolled participants and their kin for the study. The study was approved by Ethical Committee of Central India Institute of Medical Sciences (CIIMS), Nagpur.

STATISTICAL ANALYSIS

Demographic and baseline characteristics among stroke patients admitted within 24 hours and after 24 hours poststroke occurrence were analysed using chi square test. Adjusted odds ratio for analysis of long term outcome was studied using multivariate analysis by adjusting confounding factors age, sex, diabetes, hypertension, smoking, Alcohol, mRS score, and admission time. Kaplan-Meier analysis was performed to determine the long term survival in early and late referrals post discharge. ITIH4, NSE and S-100 ββ on admission and discharge in serum samples of improved and expired/ dependent AISpatients admitted within 24 hours and after 24 hours were compared using student t-test. P<0.5 was considered statistically significant for all analysis.

RESULTS

Baseline data of 104 participants with stroke in two different groups are given in [Table/Fig-3]. Among 104 participants, majority of cases (n=66) were more than 50 years of age with their ratio slighter greater in late referral cases (68%) than those admitted earlier (58%). Occurrence of stroke cases was more in males (n=73) than females (n=31) among both groups of patients. No significant impact of factors like diabetes, hypertension, IHD, smoking, alcohol was observed between early and late referrals cases admitted with stroke to CIIMS. Around 21% of early admission cases underwent thrombolytic treatment which was significant (p< 0.05) compared to 0% of cases that were admitted after 24 hours. Ratio of stroke severity observed was more in early admission cases (22%) than those of late referral cases (12%). To study prognostic impact of admission time on post

Baseline Characteristics	Admission within 24hours (n=48)	Admission after 24 hours (n=56)	p value
Age			
≤ 50 years (n = 38)	20 (42%)	18 (32%)	0.621
> 50 years (n = 66)	28 (58%)	38 (68%)	0.75
Sex			
Male (n=73)	33 (69%)	40 (71%)	0.977
Female (n=31)	15 (31%)	16 (29%)	0.989
Hypertensive			
Yes (n=70)	33 (69%)	37 (66%)	0.978
Diabetes			
Yes (n=29)	12 (25%)	17 (30%)	0.265
Ischemic heart diseases			
Yes (n=5)	2 (4%)	3 (5%)	0.849
Cardiac disease			
Yes (n=3)	3 (6%)	0	0.209
History of smoking			
Yes (n=8)	5 (10%)	3 (5%)	0.599
History of alcohol			
Yes (n=11)	6 (13%)	5 (9%)	0.831
Past History of Stroke			
Yes (n=6)	1 (2%)	5 (9%)	0.321
Thrombolysis treatment given			
Yes (n=10)	10 (21%)	0	0.003
Decompression surgery			
Yes (n=3)	3 (6%)	0	0.209
Disability Score on admission#			
Severe (n=14)	9(22%)	5(12%)	0.322
Moderate (n=44)	20(49%)	24(60%)	0.91
Mild (n=23)	12(29%)	11 (28)	0.922

[Table/Fig-3]: Baseline characteristics of study population (n=104).
Note: *out of 64 Patients, #Out of 81 patients

stroke occurrence, outcome studies were carried out depending on level of improvement and death/dependency at time of discharge and at 12th and 18th month post discharge. [Table/Fig-4a] shows hospital outcome in early and late referral AIS cases admitted to CIIMS. The hospital outcome was available among 104 cases of AIS. Although not significant, dependency rates were comparatively more among late referrals (18%) as compared to early referrals cases (6%). Similarly there were more cases that showed improvement at the time of discharge in early referrals (89%) as compared to that observed in late referrals (77%).

[Table/Fig-4b,c] shows long term outcome in early and late referral at 12th and 18th month post hospital discharge. The long term outcomes were available in 60 cases at 12 month and 56 cases at 18th month out of overall 104 cases. No significant results were obtained in terms of long term outcome at 12th and 18th month respectively. Although percentage of cases that remained improved post discharge were more in early referrals (57% & 75%) than late referrals (48% & 67%) at 12th and 18th month respectively. On the contrary there were comparatively more cases that became dependent after improvement post hospital discharge in late referrals (32%) compared to early referrals (23%).

Survival analysis was carried out in both early and late referrals using Kaplan-Meier analysis [Table/Fig-5a]. No significant difference in survival time was observed in AIS patients of both the groups. Mean survival time was 17.882 ± 0.73 among late referrals compared to 18.754 ± 0.74 observed in early referral cases over 12th and 18th month outcome period [Table/Fig-5b].

Multivariate analysis showing adjusted and unadjusted odds ratio of dependent and/expired outcome in early and later referrals AIS cases is depicted in [Table/Fig-6]. After adjusting for confounders, no significant impact was observed in short term outcome (AOR

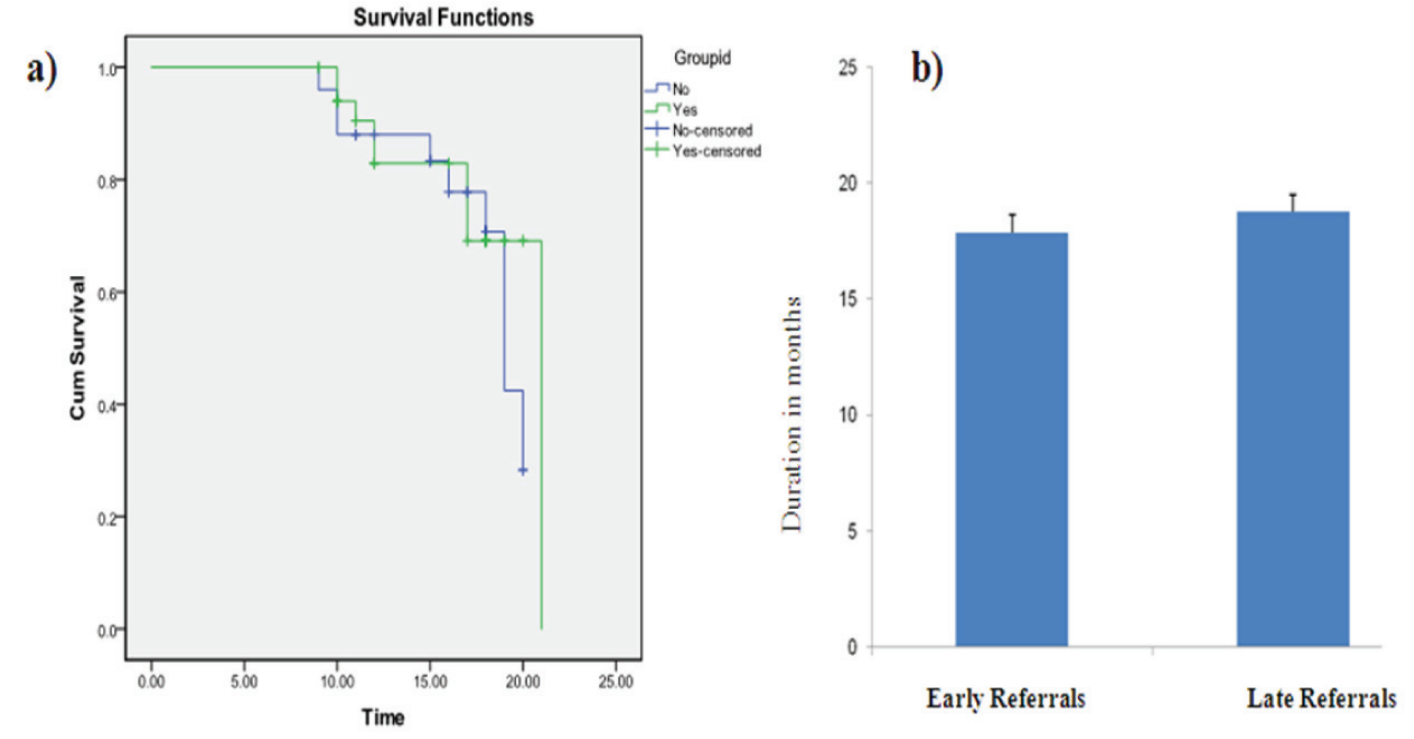
Characteristics	n(%) 104 (100)	Admission within 24hours n=48 (%)	Admission after 24 hours n=56 (%)	Difference	95% CI	Chi-square	DF	Significance level
Duration in the hospital (Days)								
≤ 15	87 (84)	37 (77%)	50 (89%)	0.12	-2.5% to 26.8%	1.897	1	p = 0.1684
>15	17 (16)	11 (23%)	6 (11%)	0.12	-2.5% to 26.8%	1.897	1	p = 0.1684
Hospital Outcome								
Improved	87 (84)	43 (90)	44 (79)	0.11	-3.5% to 24.6%	1.587	1	p = 0.2078
Dependent	13 (12)	3 (6)	10 (18)	0.12	-1.1% to 24.6%	2.4	1	p = 0.1213
Expired	4 (4)	2 (4)	2 (4)	0	-10.1% to 9.2%	0.252	1	p = 0.6157

Characteristics	n(%) 60 (100)	Admission within 24hours n=35 (%)	Admission after 24 hours n=25 (%)	Difference	95% CI	Chi-square	DF	Significance level
Improved #/Improved*	32 (53)	20 (57)	12 (48)	0.09	-15.6% to 32.3%	0.182	1	p = 0.6698
Improved #/Dependent*	16 (27)	8 (23)	8 (32)	0.09	-12.9% to 31.4%	0.231	1	p = 0.6308
Improved #/Expired*	4 (7)	3 (9)	1 (4)	0.05	-11.6% to 19.3%	0.056	1	p = 0.8130
Dependent #/Improved*	0	0	0					
Dependent #/Dependent*	8 (13)	4 (11)	4 (4)	0.05	-12.3% to 24.8%	0.032	1	p = 0.8588
Dependent #/Expired*	0	0	0					

Note: # Hospital outcome; *12 month outcome.

Characteristics	n 56 (100)	Admission within 24 hours n=32 (%)	Admission after 24 hours n= 24 (%)	Difference	95% CI	Chi-square	DF	Significance level
Improved*/Improved$	40 (71)	24 (75)	16(67)	0.08	-14.9% to 31.2%	0.128	1	p = 0.7207
Improved*/Dependent$	0	0	0					
Improved*/Expired$	0	0	0					
Dependent*/Improved$	0	0	0					
Dependent*/Dependent$	9 (16)	4 (13)	5 (21)	0.08	-11.5% to 29.1%	0.189	1	p = 0.6634
Dependent */Expired$	7 (13)	4 (13)	3 (13)	0	-17.8% to 20.1%	0.161	1	p = 0.6881

[Table/Fig-4a-c]: Hospital and long term outcome among early and late referral AIS stroke cases (A: Hospital Outcome) (B:12 Month Outcome) (C: 18 Month Outcome) *12 month outcome $ 18 month outcome



[Table/Fig-5]: a) Line graph shows Kaplan-Meier estimates of cumulative risk of second stroke in those with an ischemic stroke and history b) shows mean and median survival time observed in short term and at 12th and 18th months post hospital discharge among early and late referrals cases with AIS. b) Kaplan-Meier estimates of cumulative risk of mortality among the early (blue) and late (Green) referrals cases with AIS. b) mean and median survival time observed in short term and at 18th months post hospital discharge among early and late referrals cases with AIS

Outcome	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Outcome at discharge		
Dependent/Expired (n=17)	1.26 (0.43 - 3.83)	1.811 (0.271 - 12.113)
Long term outcome		
At 12 months		
Dependent/Expired (n=20)	1.22 (0.40 - 3.69)	1.089 (0.310 - 3.821)
At 18 months		
Dependent/Expired (n=16)	1.48 (0.45 - 4.95)	1.419 (0.361 - 5.574)

[Table/Fig-6]: Multivariate analysis showing adjusted and unadjusted odds ratio of dependent and/expired outcome in early and later referrals AIS cases

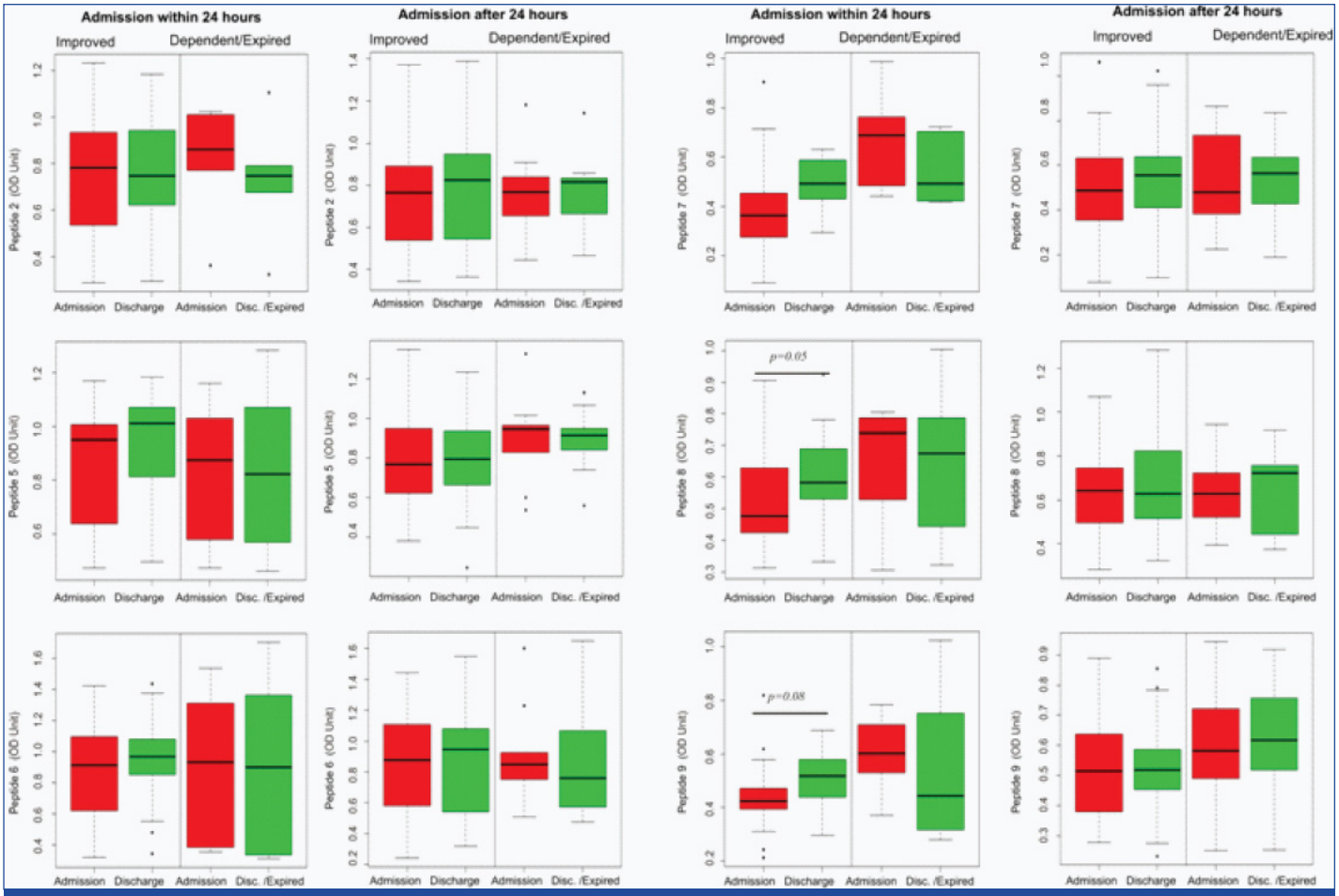
95% CI 1.811,) and long term outcome at 12 months (AOR 95% CI 1.089) and 18 months (AOR 95% CI 1.419) among early and late admission AIS cases.

To study utility of serum biomarkers for prognosis along with severity of infection in AIS patients, stroke markers like NSE and S-100 ββ along with ITHIH4 peptides were evaluated in both early and late referrals. Both groups of patients were further categorized as improved, dependent/ expired based on hospital outcome from point of admission to discharge. Analysis of 9 ITIH4 peptides revealed significant association of peptides 5 and 7 with level of improvement in early referral cases [Table/Fig-7] Moreover, no such association between peptides was observed in both improved and expired cases of late referrals group. Similarly, estimation of markers NSE and S-100 ββ showed significant association between their serum levels along with level of improvement in early referrals compared to late referrals. Improved cases in early referrals group had significantly low levels of NSE and S-100 ββ compared to expired cases. In contrast, levels of NSE and S-100 ββ were found to be significantly raised in improved late referrals cases on admission, thereby showing impact of early admission on prognostic outcome in AIS stroke. Levels of both NSE and S-100 ββ were found to be significantly raised in expired cases similar to that observed in dependent cases in early referrals group [Table/Fig-8].

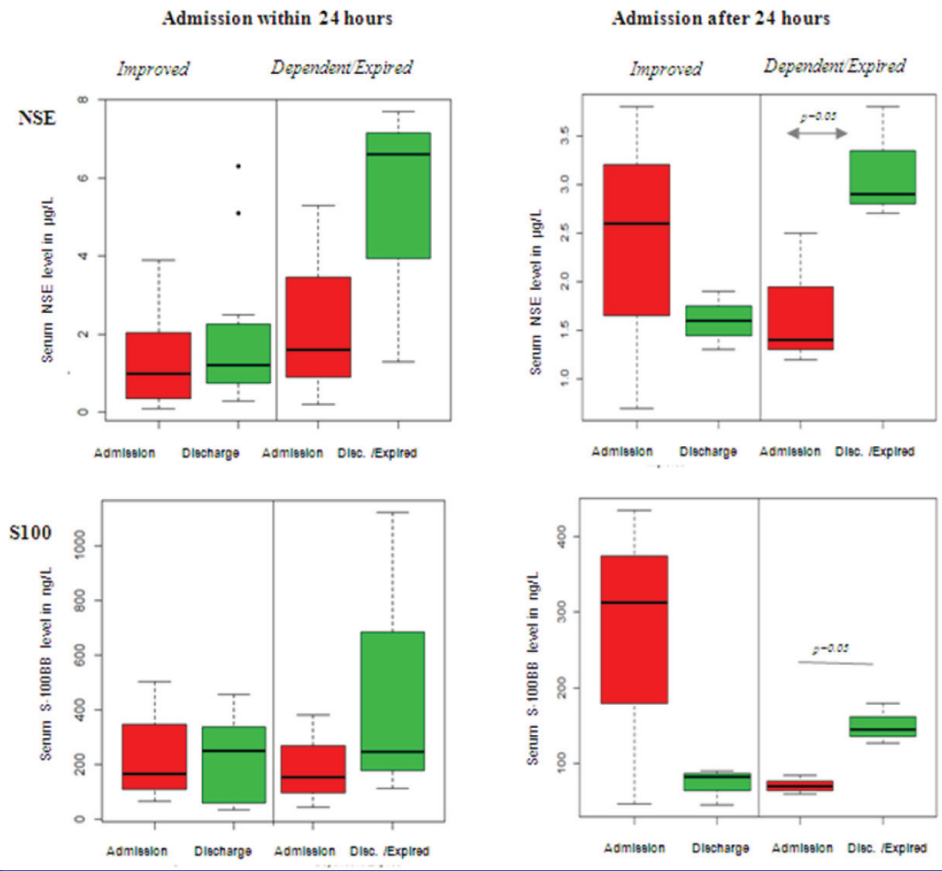
DISCUSSION

Time interval from onset of stroke to treatment plays a vital role in AIS patients. Admission of patients within window period post occurrence of stroke has been linked with better treatment, outcome and recovery rates [9]. The present study investigated impact of admission time on severity of stroke outcome among patients admitted within 24 hours and after 24 hours after stroke occurrence. 104 patients admitted to neurology department of CIIMS, were categorized into early and late referrals based on arrival time. Biomarkers of stroke: NSE, S-100 ββ and ITIH4 were also investigated in patients of both groups to determine utility of the such markers in prediction of stroke severity with respect to different admission time.

Baseline data of admitted patients showed more number of cases in older age group in both categories of patients, with males predominating than females, which is in accordance with earlier studies [10,11]. Similarly our results are in agreement with other reports which suggest better eligibility for thourombolysis treatment and surgical interventions in patients that arrived earlier as compared to late admission cases. In our study, almost 21% early referrals cases underwent thrombolysis treatment compared to late referrals where none of the cases underwent the same. However, no significant difference among risk factors like alcohol, smoking, and diabetes were observed in either category of patients. Multivariate analysis also revealed no significant impact of above confounders on outcome of AIS in both study groups. Major reason for this may be possibly due to less number of overall cases with smoking, diabetes and alcohol recruited in either group.



[Table/Fig-7]: Represents Box plots for absorption values of nine ITIH4, on admission and discharge in serum samples of improved and expired/dependent AIS patients admitted within 24 hours (n=45) and after 24 hours (n=54) respectively. Results were expressed as median values with lower and upper quartiles. Whiskers displayed non-outliner maximal and minimal values



[Table/Fig-8]: Represents Box plots for absorption values of NSE and S-100 ββ post admission and discharge in serum samples of improved and expired/ dependent AIS patients admitted within 24 hours (n=45) and after 24 hours (n=54) respectively. Results were expressed as median values with lower and upper quartiles. Whiskers displayed non-outliner maximal and minimal values

Studies have shown HTN to be one of the major risk factors linked to AIS outcome [12]. From our observation, although number of patients with hypertension were more or less similar in both groups, hypertension accounted for almost 71% of overall stroke cases in population under study.

Early treatment with recombinant tissue plasminogen activator (rtPA) improves functional outcome by effectively reducing disability and dependency [13]. In our study, we observed similar trend wherein despite higher stroke severity in early referrals cases, we observed marginally better rates of recovery in them in terms of hospital outcome as compared to late referrals who had less severity. The outcome results at long term follow up were compared to that at time of discharge during hospital. Although not significant, there were more number of early referrals that showed improvement at time of discharge, with low conversion rates into dependency during long term outcome. Major reasons for lack of significance can be lesser number of follow up cases in long term outcome. Similarly, it was not possible to trace outcome of all cases in each follow due to unavailability of patients.

Long term outcomes carried out at 12th and 18th post discharge indicated no significant difference in death /dependency. Kaplan-Meier estimates of cumulative risk of second stroke in patients after discharge indicated similar survival rates in both early and late referral cases. These results suggest that although admission time may play a vital role in terms of therapeutic interventions in short term outcome, it may have little impact on death/dependency in terms of long term stroke outcome.

Limited evidences are available on impact of admission time on functional outcome in AIS patients. Other studies in these regard suggest variable impact of admission time in AIS cases. Study done by Haeusler et al., on impact of admission time on non working hours after thrombolysis for stroke showed limited impact of admission time of short term outcome in stroke [14]. Another study by Salter et al., suggests better functional gains and shorter stay in patients admitted earlier after 30 days post stroke occurrence [5]. Despite debatable evidences on admission time on stroke outcome, early admission to stroke care unit is generally encouraged. In the current study, our results suggests limited impact of admission time on stroke outcome and recovery in both cases however as discussed earlier, patients have better chances of recovery despite high stroke severity if admitted earlier due to greater possibility of undergoing tPA therapy. However, further studies in this regard needs to be carried out to further justify the impact on admission time on short and long term outcome in AIS patients.

Another aspect of the study was to evaluate biomarkers of neuronal damage in AIS patients to study impact of time of admission on level of prognosis in both patient groups. Both early and late referrals were further categorized as improved and dependent/ expired based on mRS scale at the time of discharge. Stroke markers, NSE, S-100 ββ and ITIH4 peptides were evaluated at time of admission as well as discharge in improved and cases that were dependent or expired. Analysis showed significant association of ITIH4 peptides with level of improvement in early referrals cases. Similarly improved cases in early referrals were associated with low NSE and S-100 ββ levels thereby showing better prognosis. On the contrary, improved cases in late referrals were associated with similar ITIH4 levels at admission and discharge. Although improved late referrals were associated with low NSE and S-100 ββ, they showed comparatively higher levels of both markers on admission, compared to improved cases in early referral group, suggesting that late admission is associated with more neuronal damage and poor prognosis. The results also suggest that although baseline data showed limited impact of time of admission on post stroke outcome, analysis of stroke markers provide significant information on prognostic outcome and stroke severity associated with time of admission in AIS patients.

NSE is a dimeric isoenzyme of the glycolytic enzyme enolase and is found mainly in the neurons and cells of the neuroendocrine system [15]. Thus high levels of NSE in serum/CSF often indicate neuronal

damage in case of neurological disorders such as stroke [16]. Some studies have shown a positive correlation between NSE levels and infarct volume in patients of AIS [17,18]. S100 ββ is a calcium-binding protein expressed mainly in human astroglial cells. Because astroglia are as sensitive as neurons to hypoxia, serum S100 ββ levels have the potential to be a surrogate marker for neuronal damage and damage to the blood-brain barrier [19]. Our results are in agreement with above evidences, wherein improved cases in late referrals had higher NSE and S-100 ββ levels on admission compared to early referrals group, suggesting that late admission of AIS cases are associated with more neuronal damage than cases which are admitted earlier.

Another marker that we analysed was ITIH4, which is 120kDa anti- inflammatory marker earlier reported by our group. Studies by Kashyap P et al., have shown ITIH4 to be specifically expressed in serum of healthy patients and down regulated in AIS cases [8]. In the present study, peptides of ITIH4 were designed and evaluated in patient groups. Results suggests a significant association of ITIH4 levels with level of improvement especially in cases admitted earlier which is in agreement with our earlier published observation. Moreover, results suggests that peptides based assay of stroke markers may play an important role in stroke diagnosis by offering dual advantage of being cost effective and an alternate to antigen based assay for stroke prognosis during hospital outcome.

LIMITATIONS

The present study had certain limitations. Major limitation includes the low sample size. Another limitation is the lack of follow up in all the patients in prediction of long term outcome. The reason for this is the change of address and contact numbers of majority of patients' admitted in our institute, since most of them were from outside the state which limited their recruitment for follow up study. Lack of estimation of stroke markers in follow up samples including at hospital discharge was also one of the limitations associated with the study. However, lack of such studies carried out in Central India in this regard warrants an edge over other studies despite the number of limitations associated with it.

CONCLUSION

To conclude, early admission of AIS patients are associated with better hospital outcome and low levels of NSE, S-100 ββ and ITIH4 however it has no major impact on long term outcome in patients. Further studies focusing on prognostic aspect with large sample size with adequate follow up is therefore needed to justify the results.

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