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EVALUATION OF DEMOGRAPHIC AND CLINICAL FACTORS AFFECTING DISEASE OUTCOME AFTER ACUTE ISCHEMIC STROKE IN PATIENTS ADMITTED TO A TERTIARY CARE CENTRE IN INDIA

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ABSTRACT

In the present study, we assessed the demographic and clinical factors that affect mortality and functional outcome in acute ischemic stroke (AIS) patients at the time of hospital discharge and at 18 months after discharge. The study was carried out in a total of 104 AIS patients admitted to Central India Institute of Medical Sciences (CIIMS), Nagpur, a tertiary care centre in Central India. Relationships between nominal variables in the demographic and baseline clinical characteristics among AIS patients in the categories of hospital outcome and long-term outcome were analyzed. Ischemic heart disease (IHD) and hypertension were found to be an associated risk factor for hospital death or dependency and clinical outcomes at 18 months after discharge in AIS patients respectively. 85% of older AIS patients (>50 years) who had an improved outcome at hospital discharge were found to have dependent/expired outcome at 18-months follow-up whereas 75% of younger AIS patients (< 50 years) who were discharged with dependent hospital outcomes were found to have improved outcome at 18 months follow-up. The result demonstrates a significant association of variables such as IHD, age, and HTN with the functional outcome at discharge time and at 18 months follow-up in AIS patients.

KEYWORDS : Neurological Disorder, Acute Ischemic Stroke, Prognosis, Biomarkers, Itih4 Protein

Acute ischemic stroke (AIS) is a clinical condition accompanied by oxidative stress and inflammation (Kashyap et al., 2009; Shenhar-Tsarfaty et al., 2008; Weinberger et al., 2006). Very few studies have been done to investigate the factors influencing outcome after AIS. It was reported that information on demographic and clinical factors such as age, gender, severity, etiology of injury etc. may help in the prognosis for recovery in AIS patients (Joseph and Rhoda, 2013). Another study by Feigenson et al., 1977 has also reported that medical and functional prognostic indicators may adversely affect the functional outcomes in AIS patients (Feigenson et al., 1977). AIS not only cause long term disability in patients but also render them dependent on others for assistance with daily routine activities (Abraha et al., 1997; Fassbender et al., 1997). Identification of clinical and demographic factors associated with unfavorable hospital outcome as well as post-discharge outcome in AIS patients may aid health professionals in planning their treatment during hospitalization and rehabilitation post-hospital discharge which could ultimately reduce the mortality and morbidity caused due to AIS.

Over the past few decades, analyses of brain damage biomarkers in a variety of central nervous system (CNS) disorders have attracted the attention of several researchers (Kashyap et al., 2009; Abraha et al., 1997; Fassbender et al., 1997; Missler et al., 1997; Büttner et al., 1997). In a previous work reported by our laboratory, ITIH4 (levels were estimated during the diagnosis and prognosis of AIS patients (Kashyap et al., 2009).

In the present study, we assessed the demographic and clinical factors that affect mortality and functional outcome at the time of hospital discharge and at 18 months after discharge in AIS patients who were admitted to Central India Institute of Medical Sciences (CIIMS), Nagpur a tertiary care centre in Central India. We also estimated serum ITIH4 levels and determined its association with functional outcome in AIS patients.

MATERIALS AND METHODS

Study Subjects

The present study has been carried out in a total of 104 patients. All the patients were selected from in-patient department and intensive care unit (ICU) of CIIMS, Nagpur, India. Diagnosis of AIS was based on the WHO

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definition of stroke such as rapidly developing signs of focal (or global) disturbance of cerebral function lasting >24 hrs (unless interrupted by surgery or death), with no apparent non-vascular cause. In addition, history, neurological examination and computerized tomography (CT) were also recorded. All patients were admitted to the ICU where the ambient temperature was maintained between 20-25°C. The protocol for this study was reviewed and approved by the Institutional Ethics Committee of CIIMS.

A formal consent was taken from each of the participant included in the study and a predesigned proforma based questionnaire was recorded and stored for all AIS patients. Demographic and clinical factors such as age, gender, risk factors and behavioral factors, past history of stroke, severity of stroke and duration of hospital stay for each patient was recorded. Smoking was defined as current use of > 1 cigarette per day. Diabetes mellitus (DM) was defined as receiving oral hypoglycemic agents/insulin treatment. Hypertension (HTN) was recorded based on antihypertensive treatment.

Neurological deficit was assessed using the National Institute of Health Stroke Scale (NIHSS) score at the time of admission. AIS patients were classified into three groups based on NIHSS score; these were minor stroke (NIHSS score 1-6), moderate stroke (NIHSS score 7-18), and severe stroke (NIHSS score 19-42). Similarly, modified Rankin Scale (mRS) was used for evaluation of outcome in AIS patients at the time of hospital discharge and then at 18 months follow-up after discharge. Out of 104 patients, 18 months follow-ups were obtained for 60 AIS patients.

In addition, the AIS patients were also categorized into following three groups based on their hospital outcome and follow-up responses 18 months after discharge. These include Group A: Discharge with improvement/improved during 18 months follow-up (n=32); Group B: Discharge with improvement/Dependent / expired during 18 month follow-up (n=20); Group c: Discharge with Dependency/ Improved during 18 months follow-up (n=8).

Treatment

All AIS patients received antiplatelet agents (aspirin 150 mg and Clopadrigel 75 mg) once a day. Out of 104 AIS patients, 10 were thrombolyzed using intravenous recombinant tissue plasminogen activator (rtPA) while 3 patients were treated with decompressive hemicraniectomy and duroplasty for malignant middle cerebral artery syndrome.

Exclusion Criteria

AIS patients with hemorrhagic stroke, brain malignancies, transient ischemic attack, patients who underwent brain operation; patients who presented with severe systemic disease, dementia, psychiatric disease, active infection and patients who took discharge against medical advice were excluded from the study.

Sample Collection

For estimation of ITIH4, venous blood samples were collected from AIS patients at 0 hrs (i.e. at the time of admission). Blood was allowed to clot and after centrifugation (1000 ×g for10 min) the serum was separated and stored at −20°C until it was used.

Designing and Synthesis of Peptides and Anti-Peptides 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 method (Kolaskar and Tongaonkar, 1990) by using online software titled "Molecular Immunology Foundation-Bioinformatics". These antigenic peptide sequences were then subjected to multiple sequence alignment using NCBI BLAST to obtain the sequence similarities with other nonredundant protein database sequences. Based on the results of the blast analysis, a total of nine antigenic sequences of ITIH4 were selected. These peptide sequences were then sent for antibody production to GenicBio lab, Shanghai, China.

ITIH4 Estimation

An in house ELISA method was employed for detection of ITIH4 in serum of AIS patients. Briefly, 96 wells microtiter plate 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 min of incubation, 100µL of primary antibody

(ITIH4 anti-peptide antibody raised in rabbit) was added and further incubated for 45 min. For colour development, 100µL anti-rabbit HRP conjugated secondary antibody (dilution 1:10,000) was added and incubated at 37°C for 45 min. The reaction was stopped with 100µl H_2SO_4 (2.5 N) and intensity of colour developed was measured at 450nm using an enzyme linked immunosorbant assay (ELISA) reader.

Statistical Analysis

Relationships between nominal variables in the demographic and baseline characteristics among AIS patients in the categories of hospital outcome and long-term outcome were analyzed using chi-square test. The data on continuous variables was summarized in terms of mean and standard deviation and statistical significant difference in the variable means between groups was determined using the t-test. Significant difference in the mean levels of ITIH4 was also determined using the t-test. The level of significance was set at $P<0.05$. Adjusted odds ratio for analysis of a long-term outcome was studied using multivariate logistic regression analysis by adjusting confounding factors like age, sex, admission time within 24 hr, DM, HTN, alcohol, and thrombolysis. All statistical analyses were performed using R-programming language (version: 3.0.0).

RESULTS

In the present study, the association of outcome after AIS with clinical, demographical and laboratory parameters were investigated in patients admitted to the Neurology Department of CIIMS, Nagpur. AIS patients were classified in different groups depending on hospital outcome at the time of discharge and follow-up point of 18 months after discharge as shown in Figure 1.

Table 1 depicts association of demographic and clinical characteristics of AIS patients with hospital outcome. Out of 104 AIS patient, 87 patients were discharged with improvement, 13 were discharged with dependent outcome and 4 patients expired. Majority of cases (67%) belonged to older age group (>50 years) in the improved category, whereas, similar frequency of age group was observed in the Dependent/Expired group. The numbers of AIS cases were more in males (69% and 76%) as compared to females in both the groups respectively. The characteristics of patients in the improved or dependent/expired category showed a significant difference between the two while considering the presence of IHD. However, no significant associations were observed with respect to other characteristics like DM, HTN, smoking, alcohol etc.

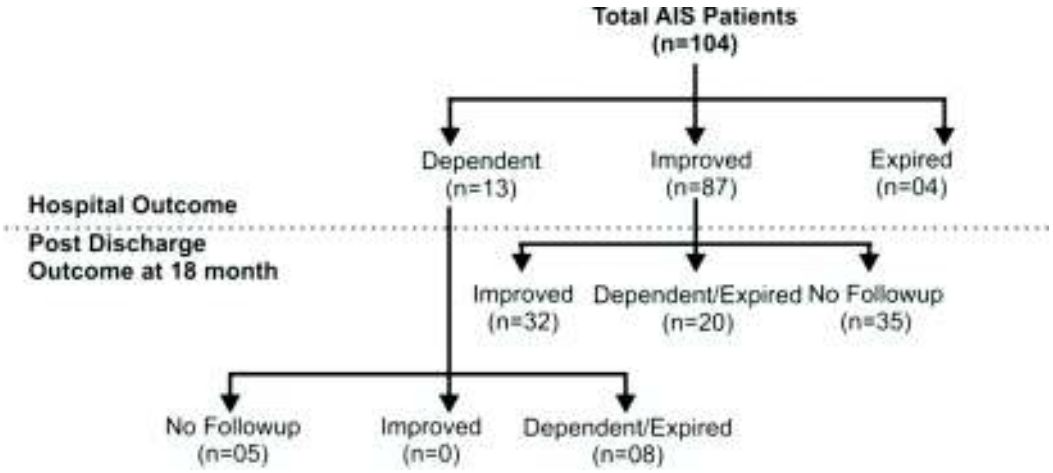

Figure 1: Flow Chart Depicting Number of Cases Under Different Outcome Categories at The Time of Hospital Discharge and Post Discharge Follow-up Outcome at 18 Months.

Table 1: Association of Demographic and Clinical Characteristics of Ais Patients with Hospital Outcome

Characteristics	Improved (n=87)	Dependent/Expired (n=17)	P- value
Age			
≤ 50 years (n = 38)	29 (33)	9 (53)	0.2076
> 50 years (n = 66)	58 (67)	8 (47)	
Sex			
Male (n=73)	60 (69)	13 (76)	0.7422
Female (n=31)	27 (31)	4 (24)	
Admission within 24 hrs			
Yes (n=48)	41(47)	5 (29)	0.2712
Duration in the hospital (days)			
≤ 1 5 (n=87)	72 (83)	15 (88)	0.8415
>15 (n=17)	15 (17)	2 (12)	
Associated risk factor			
Hypertensive			
Yes (n=70)	58 (67)	12 (71)	0.968
Diabetes			
Yes (n=29)	25 (29)	4 (24)	0.9010
Ischemic heart diseases			
Yes (n=5)	2 (2)	3 (18)	0.0248
Cardia c disease			
Yes (n=3)	3 (3)	0 (0)	0.9010
History of smoking			
Yes (n=8)	7 (8)	1 (6)	0.8299
History of alcohol			
Yes (n=11)	9 (10)	2 (12)	0.8510
Past History of Stroke			
Yes (n=6)	6 (7)	0 (0)	0.5758
Thrombolysis			
Yes (n=10)	7 (8)	3 (1 8)	0.4073
Decompression surgery			
Yes (n=3)	84 (97)	17 (100)	0.9010
Disability Score on admission			
Severe (n=14)	20 (23)	3 (18)	0.895*
Moderate (n=44)	38 (44)	6 (35)	
Mild (n=23)	19 (22)	4 (24)	
NA (n=23)	10 (11)	4 (24)	

*Excluding Cases Under NA Category, P Value <0.05 was Considered as Significant

The long-term outcome of the patients was assessed after 18 months and characteristics were correlated as shown in Table 2. The outcome was available for 60 cases of AIS based on clinical condition of patients of the improved and dependent categories at 18 months follow-up. The groups were classified as Group A: Improved/Improved (n=32), Group B: Improved/ Dependent or Expired (n=20) and Group C: Dependent/ Improved (n=8) depending on outcome at discharge and at 18 months. Patients in Group A and Group B belonged to older age group (> 50 years) whereas majority of patients in the Group C category were in the younger age group of ≤ 50 years (P=0.0086). HTN was found to be an associated risk factor affecting clinical outcomes determined at 18 months follow-up of AIS patients. Significantly, more numbers of patients were hypertensive who became dependent or expired than those who improved at 18 months. When multivariate logistic-regression analysis was done by

Table 2: Association of Demographic and Clinical Characteristics of AIS Patients With Post Discharge Follow-Up Outcome Collected at 18 Months

	Outcome: at Discharge / at 18 months (*n*=60)			
Characteristics	**Improved/Improved (n = 32)**	**Improved/Dependent or Expired (n = 20)**	**Dependent/Improved (n = 8)**	**P-value**
Age				
≤ 50 years (n = 19)	10 (31)	3 (15)	6 (75)	0.0086
> 50 years (n = 41)	22 (69)	17 (85)	2 (25)	
Sex				
Male (n=42)	23 (72)	13 (65)	6 (75)	0.8241
Female (n=18)	9 (28)	7 (35)	2 (25)	
Admission within 24 hrs.				
Yes (n=35)	20 (63)	11 (55)	4 (50)	0.7601
Duration in the hospital (Days)				
≤ 15 (n=87)	26 (81)	16 (80)	8 (100)	0.3946
>15 (n=17)	6 (19)	4 (20)	0	
Associated risk factor				
Hypertensive				
Yes (n=42)	18 (56)	18 (90)	6 (75)	0.0336
Diabetes				
Yes (n=23)	11 (34)	10 (50)	2 (25)	0.3743
Ischemic heart diseases				
Yes (n=3)	1 (3)	1 (5)	1 (13)	0.5532
Cardiac disease				
Yes (n=2)	2 (6)	0	0	0.4045
History of smoking				
Yes (n=2)	2 (6)	0	0	0.4045
History of alcohol				
Yes (n=7)	4 (13)	2 (10)	1 (13)	0.9604
Past History of Stroke				
Yes (n=4)	2 (6)	2 (10)	0	0.6258
Thrombolysis				
Yes (n=7)	4 (13)	2 (10)	1 (13)	0.9604
Decompression surgery				
Yes (n=2)	2 (6)	0	0	0.4045
Disability Score on admission				
Severe (n=7)	2 (6)	4 (20)	1 (13)	0.5468
Moderate (n=24)	13 (41)	9 (45)	2 (25)	
Mild (n=17)	11 (34)	4 (20)	2 (25)	
NA (n=12)	6 (19)	3 (15)	3 (37)	

*Excluding Cases Under NA Category

Table 3: Multivariate Logistic Regression Analysis of Patients With Long Term Outcome on 18 Months

Characteristic	Level	Total	Outcome at: *Discharge/#18 months	
			Improved*/Dependent or Expired# (n = 20)	Dependent*/Improved# (n = 8)
Age (years)				
	≤ 50	*19*	1	1
	> 50	*41*	0.984 (0.165 - 5.872)	0.054 (0.006 - 0.481)
Sex				
	Male	*42*	1	1
	Female	*18*	1.126 (0.290 - 4.368)	0.479 (0.047 - 4.909)
Admission within 24 hr				
	Yes	*35*	1	1
	No	*25*	1.172 (0.321 - 3.277)	1.905 (0.269 - 13.481)
Diabetes				
	Yes	*23*	1	1
	No	*37*	0.829 (0.228 - 3.013)	1.905 (0.196 - 18.524)
Hypertension				
	Yes	*42*	1	1
	No	*18*	0.146 (0.022 - 0.964)	0.096 (0.010 - 0.921
Alcohol				
	Yes	*7*	1	1
	No	*53*	1.328 (0.175 - 10.062)	1.026 (0.055 - 19.247)
Thrombolysis				
	Yes	*7*	1	1
	No	53	0.625 (0.84 - 4.652)	0.786 (0.032 - 19.611)

Note: The reference category is Improved at discharge/Improved at 18 months

adjusting for improved outcome at discharge and at 18 months, it was observed that age (odds ratio 0.054; 95% CI, 0.006 - 0.481) and HTN (odds ratio 0.096; 95% CI, 0.010 - 0.921) were associated with improved outcome of patients with dependency at discharge (Table 3).

Results of ITIH4 estimation using synthetic peptide based ELISA in serum samples of AIS patients with improved and dependent or expired outcome is given in Figure 2. ITIH4 estimation using anti-peptides 1, 6, 8 and 9 showed that AIS patients with improved outcome at discharge and favorable long-term outcome (i.e., Group A: Improved/Improved) had more levels of ITIH4 in samples obtained at discharge as compared to samples collected on admission. 18 months long-term outcome was not favorable (i.e., Group B: Improved/Dependent) in patients who had equal or decreased levels of ITIH4 in discharge samples. However, lower levels of ITIH4 were found in follow-up samples of patients dependent at the time of discharge but improved after 18 months (Group C: Dependent/ Improved). ITIH4 estimation using antipeptide namely 2, 3, 4, 5 and 7 did not show differences among the three groups.

DISCUSSION

In the present study, we investigated the long-term mortality in AIS patients after their discharge from the hospital and determined the relationship between demographic variables associated with unfavourable hospital outcome and follow-up point of 18 months after discharge in 104 AIS patients admitted to neurology department of CIIMS. The level of reported AIS biomarker,

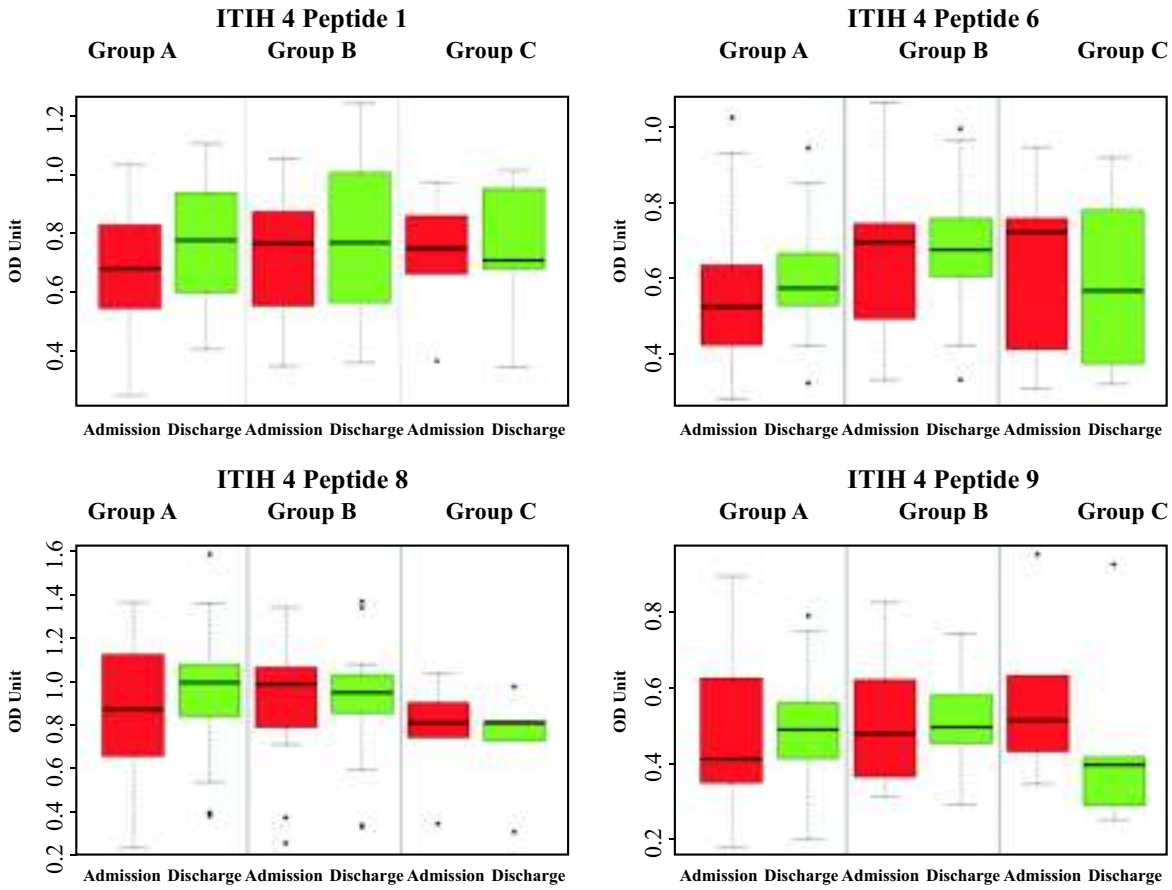

Figure 2: Levels of ITIH4 Detected by Ppeptide (1, 6, 8, and 9) in Young (n= 38) and Old (n= 66) AIS Patients with Improved and Dependent/Expired Outcome.

Note:
Group A: Discharge with Improvement/Improved During 18 Months Follow-Up;
Group B: Discharge With Improvement/Dependent or Expired During 18 Month Follow-Up;
Group C: Discharge With Dependency/ Improved During 18 Month Follow Up.

ITIH4 was estimated in the blood samples AIS patients with respect to different clinical outcomes.

The results indicate that in AIS patients with dependent/expired hospital outcome, the presence of IHD was greater in frequency than those with improved hospital outcome. The results implicate that these patients could have devastating consequences of AIS if left untreated. On the contrary, the results demonstrated insignificant correlation between variables and the functional outcome and therefore require less emphasis and less of the resources on the strategies that are implemented to address those variables. Studies have suggested that investigations of the contribution of both personal and environmental factors on the outcome of persons with AIS should be addressed in order to facilitate comparison across countries.

Males were found to have higher frequency of AIS as compared to females in this study. Sun et al., has reported that for AIS patients, the mortality in males were at a higher adjusted relative risk of death as compared to females (Sun et al., 2013). The majority (66%) of the AIS patients in the study group belonged to older age group. Ratio of AIS patients with younger age group is less (i.e. 38%) as compared to older age group but it is still higher than the values reported in the previous Indian studies (Nagaraja et al., 2009; Kaul et al., 2009) while young stroke cases are less common in the developed countries (Bonita R., 2004).

In follow-up of patients after 18 months, it was observed that majority of older AIS patients (>50 years) who had an improved outcome at hospital discharge were found to have dependent/ expired outcome whereas younger AIS patients (< 50 years) who were discharged with dependent hospital outcome were found to have improved outcome. It has been reported that older AIS patients were less likely to recover favorably (Berrouschot et al., 2005; Mouradian et al., 2005; Van Oostenbrugge et al., 2006; Sylaja et al., 2006). However, in other reports the odds of favorable outcome were comparable between both age groups (Tanne et al., 2000; Engelter et al., 2005; Chen et al., 2005). Despite the younger onset of stroke in the developing countries, literature suggests that younger survivors achieve better outcomes compared to their older counterparts, especially in function-related tasks, but not to an extent of complete independence (Kalra L, 1994).

HTN is one of the most important risk factors for stroke (Waleed MS, 2009). It was observed that HTN influenced the clinical outcome at discharge and at 18 months follow-up. It resulted in dependency or death in patients at 18 months. HTN has been reported to influence the outcome after stroke, but very limited study has been reported (Semplicini et al., 2003).

In recent years, synthetic peptide based tests have been developed for various disorders using the anti-peptides against designed peptides. In the present study, based on our earlier studies, we designed synthetic peptides corresponding to antigenic epitopes of ITIH4 for the production of anti-peptides for developing a rapid and cost-effective diagnostic and prognostic protocol for AIS patients. Earlier, in our laboratory we have identified a 120-kDa serum protein, ITIH4, which is consecutively present in serum of healthy subjects, but it was found to be either in very low level or absent in AIS patients however its level returns to normal in improved AIS patients (Kashyap et al., 2006). It is suggested that the changes in ITIH4 level in follow-up samples could predict the outcome in AIS patients as mentioned in our earlier report. There is by far no report of ITIH4 correlation with outcome at long-term follow-up of AIS patients. However, concentrations of certain pro- and anti-inflammatory markers such as interleukin (IL)-6 and (IL)-10 have been reported in early stroke periods and their association with a degree of neurological deficit and/or stroke outcome (Oto et al., 2008; Basic et al., 2008). ITIH4 estimation using anti-peptides 1, 6, 8 and 9 could predict long-term outcome in AIS patients with improved hospital outcome but not in those with dependent hospital outcome.

CONCLUSION

In conclusion, results of the present study demonstrate that, older AIS age group (> 50 years) patients are associated with increased risk for dependent/expired long term outcome after hospital discharge while younger AIS age group (≤ 50 years) patients have improved long term outcome after discharge. HTN was found to be an associated risk factor for dependent /expired long term outcome after discharge. Increased serum ITIH4 level could be a predictor of improved outcome in AIS patients.

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