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Original research article

A STUDY OF NEURON-SPECIFIC ENOLASE AS POTENTIAL BIOMARKER FOR ASSESSING THE SEVERITYAND OUTCOME IN PATIENTS WITH ACUTE ISCHAEMIC STROKE

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Abstract

Introduction: Acute ischaemic infarction is the third etiology of death and first etiology of disability across the globe. Cerebrovascular accident is an emergency condition requiring immediate intervention. The blood brain barrier compromised in patients with acute ischaemic stroke, leakage of neuro-biochemical protein markers like NSE into the peripherial circulation allow pathogenesis and prognostication of patient‘s with CVA to be weighed up additionally. The current work structured to determine the marker of brain damage, NSE in serum of patient‘s with acute ischaemic infarction as a diagnostic and/or monitoring tool for early prognosis of ischaemic stroke.

Objectives: Study of neuron-specific enolase as potential biomarker for assessing the severityand outcome in patients with acute ischaemic stroke.

Materials and Methods: The material for the present study will be collected from patients who attend the Outpatient department and Inpatient department in BLDEU‘S Shri. B.M.Patil Medical College Hospital and Research Centre, Vijayapur over a period 2 years from November 2017 to June 2019. The sample size was 94 of which 47 were acute ischemic stroke patients who were studied as cases and 47 non ischaemic stroke were taken as controls and there serum NSE, GCS, NIHSS, mRS, infarct volume were estimated and the results obtained were statistically computed.

Results: In present study, Mean NSE in cases-5.558.Mean NSE in controls-0.217. In the ROC Curve for NSE, area under ROC of NSE is100% and the optimal cut offivalue is 1.48,SENSITIVITYis100%.P-value for NSE & GCS is 0.2920.P-value for NSE & NIHSS is <0.001.P-value for NSE & mRS is <0.001.Coefficeint of correlation between NSE and infarct volume r=0.026

Conclusion: Serum NSE can be used for early diagnosis, prognosis ofiacute ischaemic stroke p atients in the settings were CT scan,MRI scan not available or patients in whom CT sca n or MRIiscan contraindicated or CT scan normal. Serum NSE test may be boon to prima ry health centres and useful to reduce morbidity and mortality associated with ischaemic stroke patients with early treatment initiation.

Keywords: NSE, CVA, GCS, NIHSS, mRS.

Introduction

Stroke, most quotidian life grieving neurological disease globally. Stroke has been known since antiquity. India is in the betwixt and between of a stroke epidemic. According to WHO, worldwide each year 15 million people suffer stroke. The incidence of stroke in Indian

population has conveyed alarming upward trend. India, stroke factsheet updated in 2012,the estimated age-adjusted prevalence rate for stroke ranges between 84/100,000 and 262/100,000 in rural and between334/100,000 and 424/100,000 in urban areas[1].

Stroke ubiquity of elderly in provincial india1.1% and metropolitan India 1.9%.Ischaemic stroke most common subtype followed by haemorrhagic and embolic stroke. In emergency, concluding and treating CVA restricted by lack of investigating tool .It is vital to make sure that patients gets thrombolysis within the treating period even if CT scan normal or not available or MRI not available or contraindicated. Intial information of neuronal damage hearld by marker like neuron specific enolase. Physiologically,NSE in blood concentration negligible compared to CSF-NSE concentration. NSE; it is a dimeric isoenzyme of the glycolytic enzyme enolase and is present principally in the neurons and cells of neuroendocrine system. In stroke, blood brain barrier disrupted. The neuro-biochemical marker like NSE release in circulation assist to evaluate pathophysiology and prognosis in patients with stroke. Till now studies concentrated over discharge & dynamics about neuron specific enolase following ischaemic stroke, principally in Cerebrospinal fluid. But, everyday analysing cerebrospinalfuid exasperating & related to complications. Thus measuring serum NSE levels facilitate frequent testing with relative low risk of complications . NSE as brain biomarker might be useful as diagnostic tool as it helps in understanding into pathophysiology of neuronal damage. In hospital where CT is not yet available, it is beneficial to have serum test for acute stroke. After acute cerebral infarction serum NSE, useful marker to predict infarct volume assesing the severity and prognostic parameters higher serum NSE levels, associated with severe weakness and deterioration as observed after 7days in CVA insinuating as biomarker in prediciting neurobehavioural outcome. The **Glasgow Coma Scale** (**GCS**) is a neurological scale furnish a objective and reliable way of recording the conscious state of a person for inceptive furthermore ensuing evaluation. A patient is weighed up against the norm of the scale, and the emanating points accord a patient score between 3 (indicating deep unconsciousness) and 15 (more widely used modified or revised scale).

The **National Institutes of Health Stroke Scale** is a contrivance objectively guage the impairment engender by a stroke, constitute of 11 items, each of which scores a specific ability between a 0 and 4. previous work.3 - modest unability. Walkable unaided. 4 - Modestly serious unability. not capable do own affairs without assistance,and assistance needed to walk.5 - Serious incapability. Have need of regular tending care and attention, bedridden, incontinent.6 - Dead. Realizing most of ischaemic stroke patients have soaring probability of morbidity due to delayed treatment. This study intends to show how neuron specific enolase in primary health care setup can be employed to diagnose ischaemic stroke and initiation of thrombolysis within therapeutic window. Correlating with clinical presentations and outcome using Glasgow coma scale, national institute of health stroke scale and modified rankin scale.

Materials and Methods:

The material for the present study will be collected from patients who attend the Outpatient department and Inpatient department in BLDEU'S Shri. B.M.Patil Medical College Hospital and Research Centre, vijayapur over a period 2 years from November 2017 to June 2019. The sample size was 94 of which 47 were acute ischaemic stroke patients who were studied as cases and 47 non ischaemic stroke were taken as controls and there serum NSE, GCS, NIHSS, mRS, infarct volume were estimated and the results obtained were statistically computed.

Method of Collection of Data:
By detailed history
Degree of disability using NATIONAL INSTITUTES OF HEALTH STROKE SCALE. ELISA BIOTIN SANDWICH BASED SERUM NSE KIT to estimate serum NSE.
Volume of infarct measured by CT scan and or MRI brain imaging. Stroke severity using GLASGOW COMA SCALE
Functional neurological outcome using Modified rankin scale.
By relevant investigations like CBC, RBS, lipid profile, ECG, blood urea, creatinine, urine examination.

Results:

Table 1: Descriptive statistics –Study Group

Variables	Minimum	Maximum	Mean	Std. Deviation
Age	35	90	62.66	11.879
NSE	0.00	20.230	5.558	5.049
GCS	3	15	13.49	3.400
NIHSS admission	0	34	9.28	7.762
MRS	1	5	4.11	0.983
GCS	4	15	13.70	2.805
NIHSS 7 days	0	24	8.30	7.339
Mrs	1	6	3.74	1.242
INFARCTVOLUME	1.4	525.0	89.423	134.5642

In present study-
1) Mean patient‘s age was 62.666 yrs Minimum age 35year and maximum age 90years.
2) Mean NSE value was 5.558.Minimum 0.00 and maximum 20.230.
3) Mean GCS at admission 13.49.minimum 3 and maximum 15.
4) Mean NIHSS at admission 9.28.minimum 0 and maximum 34.
5) Mean mRS at admission 4.11.minimum 1 and maximum 5.
6) Mean GCS at 7days 13.70.minimum 4 and maximum 15.
7) Mean NIHSS at 7days 8.30.Minimum 0 and maximum 24.
8) Mean mRS at 7days 3.74.minimum 1 and maximum 6.
9) Mean infarct volume 89.423.Minimum 1.4 and maximum 525

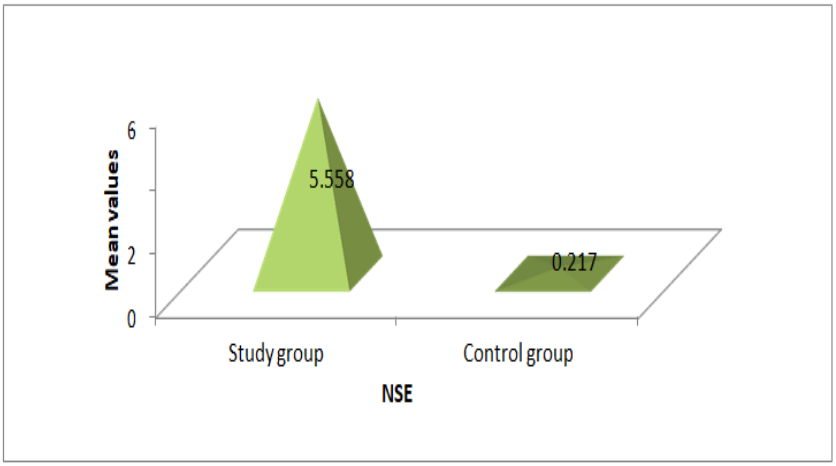
Table 2: Descriptive statistics –Control Group.

Variables	Minimum	Maximum	Mean	Std. Deviation
Age	35	82	61.19	11.009
NSE	.0000	2.789	0.2174	0.516
GCS	15	15	15.00	.000
NIHSS admission	0	0	.00	.000
MRS	0	0	.00	.000
GCS	15	15	15.00	.000
NIHSS 7 days	0	0	.00	.000
Mrs	0	0	.00	.000
INFARCTVOLUME	0	0	.00	.000

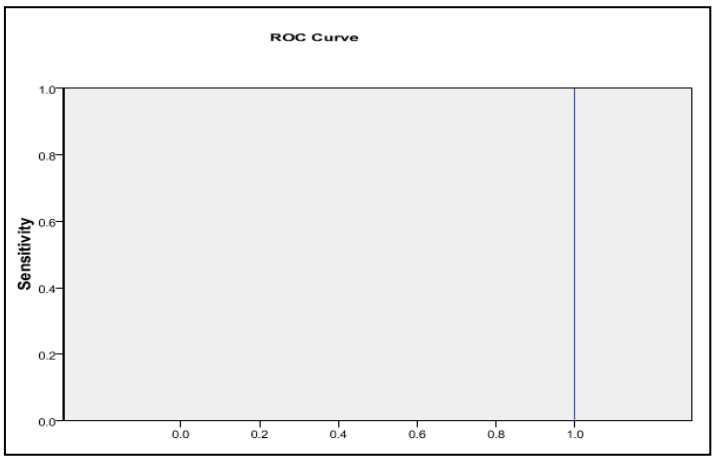
Mean age of patients in control group 61.19.Maximum age 82 and minimum age 35. Mean NSE 0.2174 ,maximum NSE 2.789 and minimum NSE 000.

Table 3: Comparison ofiNSE Between Study and Control Groups

Comparison of	Study Group		Control group		Mann WhitneyU test	P value	Remark
	Mean (Median)	±SD	Mean	±SD			
NSE	5.558 (3.69)	5.049	0.217 (0.01)	0.516	U=76.5	P<0.0001	HS
HS: Highly Significant							



In present study, levels of mean NSE higher in study group than control group with p value <0.0001 statistically significant.



Optimal cutoff value of NSE: In the ROC Curve for NSE, the Area under ROC of NSE is 100% & optimal cutoff value is 1.48 **Using our cut off values, the diagnostic test performance is**

Table 4:

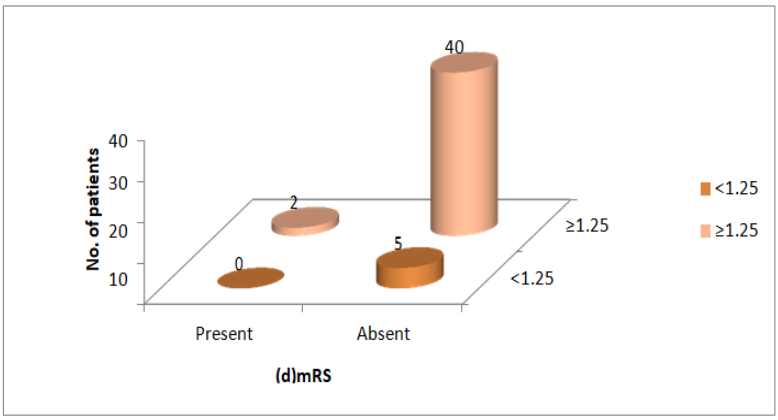
NSE	**Studygroup**	**Controlgroup**	**i squaretest**	**P value**
<1.48	5	45	X^2=68.365	P<0.0001*
≥1.48	42	02		
***: Highly significant**				

Table 5:

NSE	Study / Control
Sensitivity	**100%**
Specificity	**4%**
Positive predictive value	**11%**
Negative predictive value	**4%**

Table 6: Association between NSE and (d) mRS.

NSE	(d)mRS			
	Present	**Absent**	**Fisher's exact test**	**Remark**
<1.25	0	5	P<0.001	HS
≥1.25	2	40		
HS: Highly significant				

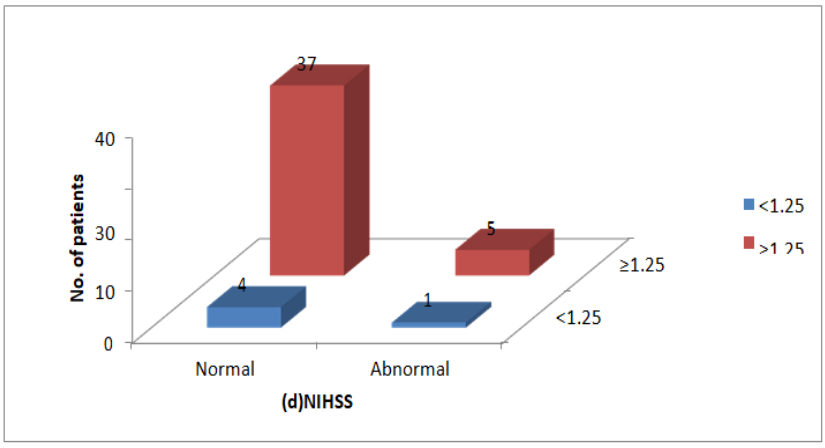


Highly significant association present between NSE and (d) mRS with p<0.001, statistically significant.

Table 7: Association between NSE and (d)NIHSS

NSE	(d)NIHSS			
	Normal	**Abnormal**	**Fisher's exact test**	**Remark**
<1.25	4	1	P<0.001	HS
≥1.25	37	5		

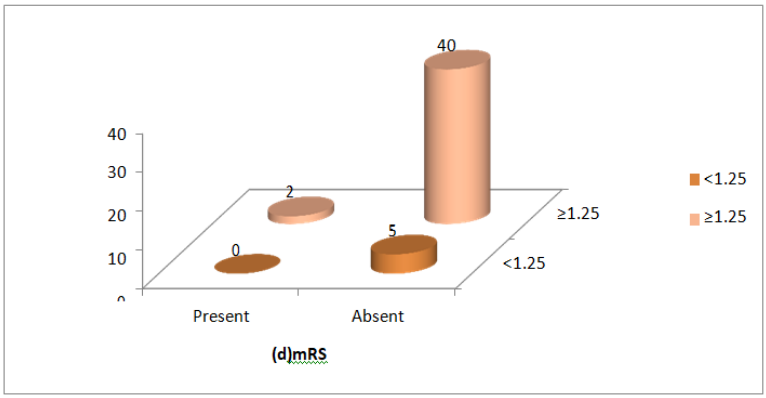
HS: Highly significant



Highly significant statistical association exists between NSE and (d)NIHSS.

Table 8: Association between NSE and (d) mRS.

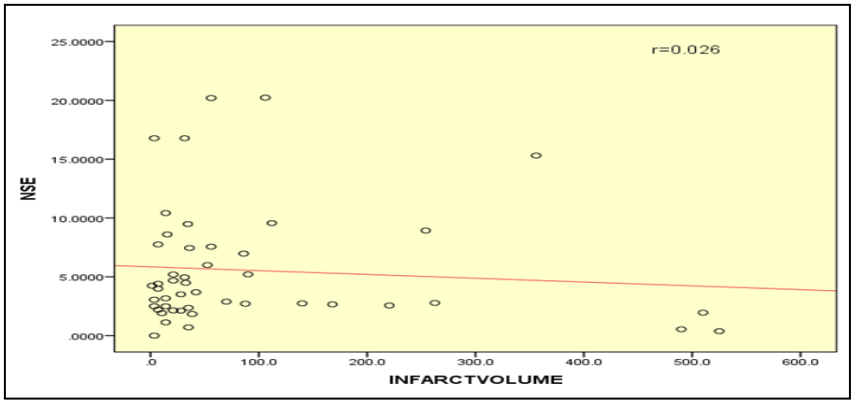
NSE	(d)mRS			
	Present	**Absent**	**Fisher's exact test**	**Remark**
<1.25	0	5	P<0.001	HS
≥1.25	2	40		
HS: Highly significant				



Highly significant association present between NSE and (d)mRS with p<0.001, statistically significant.

Table 9: Correlation between NSE and Infract Volume

Correlation between	Spearman'sCorrelation coefficient	Interpretation	P value	Remark
NSE and Infract Volume	r=0.026	Very Minute positive correlation	P=0.8642	NS



There is very minute positive correlation found between NSE at admission and infarct volume with spearman's correlation coefficient r=0.026 but statistically insignificant p-value =0.8642.

There is very minute positive correlation found between NSE at admission and infarct volume with spearman's correlation coefficient r=0.026 but statistically insignificant p-value =0.8642.

Discussion

Acute ischaemic infarction is medical crisis that endangers sufferrer's life leads to great degree of disability and death around globe. As knee jerk to infarction, neural cells releases specific neuronal markers into the blood stream. Brain damage assessed by various neuro-

biochemical markers having standard role in the diagnosis and treatment of acute infarction like NSE a neuronal form of the intra cytoplasmic Glycolytic enzyme enolase. Various researches confirmed that NSE estimated in the systemic circulation of infarct sufferers and useful marker for acute ischemic stroke.

Serum NSE levels

In present investigation,found mean levels of serum NSE in study group higher compare d to mean levels of NSE in control group,which is statistically significant with p <0.001 and consistent with studies done by Anuradha Bharosay et al [2](2012), Padalkar Ramchandra K et al [3] (2014).

Table 10:

SERUM NSE	Anuradha bharosay et al[2]	Padalkar Ramchandra k et al[3]	Present study
CASES	22.68+/-7.69	43.62+/-13.41	5.558+/-5.049
CONTROLS	7.48+/-1.52	14.55+/-12.41	0.217+/0.516

From present study,increased level of NSE seen to be related to cerebrovascular stroke.R aised NSE level during infarction because of brain ischaemia,hypoxia ,injury & convulsion.Blood – brain barrier impaired & astroglial disruption leading to leakage of NSE into the blood & cerebrospinal fluid.

Diagnostic performance of serum NSE

Ischaemic infarct leads to huge quantity of morbidity & across globe. Essential to have sufficingly sensitive marker of neural imapirement which can be estimated in blood rather in cerebrospinal fluid as blood samples taken in quick succession & independent of raised intracranial pressure compared to cerebrospinal samples.

Table 11:

Diagnostic performance of NSE	Padalakar RamchandraK et al[3]	theer H Raw i et al[4]	Hill et al[5]	Present study
Sensitivity	87.10%	85%	89%	100%

Current work diagnostic performance of Serum NSE for diagnosis of ischemic stroke analyzed. Maximum diagnostic cut off point maximizing sensitivity & specificity estimated 1.48 ng/ml,sensitivity of 100% & specificity 4%,area under Receiver operating characteristic curve for NSE 100%. Our results are totally conformity with Hill et al study.They established single examination NSE had sensitivity 89%[5].In addition ,Natheer H Rawi and Karim M Atiyah with ischemic stroke and stroke prone patients. According to their result,area under ROC curve for serum NSE significantly higher (0.960) compared to salivary NSE (0.825).Optimum cutoff level of serum NSE highest diagnostic accurancy (90%) ≥13.1μg/L.This cut-off threshold had maximum specificity (100%) & acceptable sensitivity (85%)[4].Padalakar ramchandra K et al found the optimum diagnostic cut off point maximizing the sensitivity and specificity was determined to be 40 ng/ml with a sensitivity of 87.10%and area under ROC curve for NSE 0.84[3]

Correlation between serum nse and GCS

Table 12:

Correlation between NSE and (d)GCS	Missler's et al[6]	Present study
P value	>0.05	0.2920

Current work diverged to some extent compared to previous works like GCS utilized for Assessing infarct seriousness at presentation. NIHSS usually utilized for quantifing infarct seriousness, however at latest few works utilized GCS for assessing infarct seriousness & clinicalaftereffect. Work done by González *García* et al no compelling association found between GCS and infarct seriousness at presentation[2].In present study ,found that correlation between NSE and (d)GCS worsening of severity of stroke that is GCS at admission minus of GCS at 7th day insignificantwhich is consistent with Missler *et al*,could not discovered compelling association among NSE levels & functional neurological outcome using GCS[6].

Correlation between serum nse and NIHSS(degree of disability/severity of stroke)

Table 13:

Correlation between NSEand (d)NIHSS	González García *et al*[4]	oh et al[7]	Wu et al[8]	Present study
P value	0.001	<0.001	<0.05	<0.001

In present study,found that correlation between NSE and worsening of degree of disabili ty (d)NIHSS (severity of stroke) equals to NIHSS at admission minus of NIHSS at 7th day is highly significant.–Our results are highly conformity with González-García *et al*.,they assessed functional neurological outcome by NIHSS and found a significant correlation between NSE levels and NIHSS on day 60 (P= 0.001), these authors also reported that on multivariate regression that on multivariate regression analysis, there an independent association between NSElevels and neurological outcomemeasure[4]. Oh *et al*., predicted short term prognosis using NIHSS score at day 7 and found a significant correlation between initial NSElevels and NIHSS score on day 7(P<0.001)[7]. Similar resultsobtained by Wu *et al*.,they assessed functional neurological outcome using activities of Daily Living scale and found a significant correlation between, NSE levels and outcome measure at 1 month(P<0.05), 3 months (P<0.01), and 6 months (P< 0.001)[8].

Correlation between serum nse and mRS

Table 14:

Correlation between NSE and (d)mRS	Brea et$_{al}$9	Wunderlich et$_{al}$10	Present Study
P value	<0.0001	<0.001	<0.001

–In our present study,found highly significant association among serum neuron specific enolase & worsening of functional neurological outcome (d)mRS equal to functional neurological outcome at admission minus of Functional neurological outcome at 7th day. Brea et *al*., in there study assessed functional neurological outcome using mRS at 3months, they also reported that patients with poor functional outcome (mRS >2) had significantly greater serum concentrationof NSE ($P < 0.0001$) in cases of ischemic stroke, on multivariate analysisNSE at 72 h independently associatedwith poor outcome in this study also[9]. Wunderlich *et al*.,found that serum NSE levels from 12 h onwards correlated with mRS at 3 months, with maximum association obtained for NSE at 96 h ($P<0.001$). Thus findings of our study is consistent with previous studies done[10]‖.

Correlation between NSE and infarct volume

Table 15:

Correlation between NSE and infarct volume	Brea et al[11]	Oh et al[7]	Sana zaheer et al[12]	Present study
Spearman‘s correlation coefficient (r)	0.456	0.81	0.955	0.026
Number of patients with ischaemic stroke	224	81	75	47

In present study found that very minute positive correlation exists between serum NSE with in 72hrs of onset of symptoms and infarct volume determined at day 1 in patients of ischaemic stroke.–Brea *et al*., studied 224 patients with ischemic stroke and found that NSE serum concentrations at 72 h correlated with infarct volumes determined between the 4th and 7th days (Spearman coefficient 0.456)[11].Oh *et al*., studied 81 patients with anterior circulation infarction and found a significant correlation betwe en initial serum NSE levels and infarct volume determined by T2 weighted MRI scan
($r = 0.81$)[7] .Zaheer s et al positive correlation found between concentration of NSE on day 1 and infarct volume determined by CT scan ($r =0.955$)[12] Our study differed from all previous studies can be explained by small cohort size,different timing of sampling NSE and different timing of determing infarct volume.

Conclusion

The patients admitted to BLDEU‘s Shri B.M.Patil medical college hospital and research centre,Vijayapur were selected for the present study.Total number of 94 subjects were studied which include 47 acute ischaemic stroke as cases and 47 non acute ischaemic stroke as controls.

Following are the important findings observed in the study:

1. Serum NSE levels were significantly high in cases compared to controls.
2. Serum NSE levels were absolute significant sensitive marker for diagnosis,of Ac ute ischaemic stroke patients within 72hrs of onset of symptoms.
3. Serum levels of NSE within 72hrs of onset of symptoms in acute ischaemic strok e can be of use of foreseeing severity of stroke, degree of disability
& initial functional neurological after effect.
4. Serum NSE levels may be useful marker to predict infarct volume.

In conclusion, serum NSE can be used for early diagnosis,prognosis of acute ischaemic st roke patients in the settings were CT scan,MRI scan not available,or patients in whom CT

scan or MRI scan contraindicated or CT scan normal. Serum NSE test may be boon to primary health centres and useful to reduce morbidity and associated with ischaemic stroke patients with early, treatment initiation.

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