

Original research article

A Study on Acute Ischaemic Stroke With Neuron-Specific Enolase as Potential Biomarker and the Severity and Outcome of Disease: A Hospital Based Study

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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 peripheral circulation allow pathogenesis and prognostication of patient's with CVA to be weighed up additionally. The current work structured to determine the marker of braindamage, NSE in serum of patient's with acute ischaemic infarction as a diagnostic and/or monitoring tool for early prognosis of ischaemic stroke.

Materials and Methods: The material for the present study will be collected from Department of General Medicine, Gadag Institute of Medical Sciences. Karnataka from (May 21 to June 22). 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 their 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 is 100% and the optimal cut off value is 1.48, SENSITIVITY is 100%. P-value for NSE & GCS is 0.2920. P-value for NSE & NIHSS is <0.001. P-value for NSE & mRS is <0.001. Coefficient of correlation between NSE and infarct volume $r=0.026$

Conclusion: Serum NSE can be used for early diagnosis, prognosis of acute ischaemic stroke patients in the settings where 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 a boon to primary 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 between 334/100,000 and 424/100,000 in urban areas¹.

Stroke ubiquity of elderly in provincial india 1.1% and metropolitan indi 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 heard 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 Cerebrospinalfluid. But, everyday analysing cerebrospinalfluid 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 assessing 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 comascale, national institute of health stroke scale and modified rankin scale.

Materials and Methods:

The material for the present study will be collected from Department of General Medicine, Gadag Institute of Medical Sciences, Karanataka from (May 21 to June 22).. 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.

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.

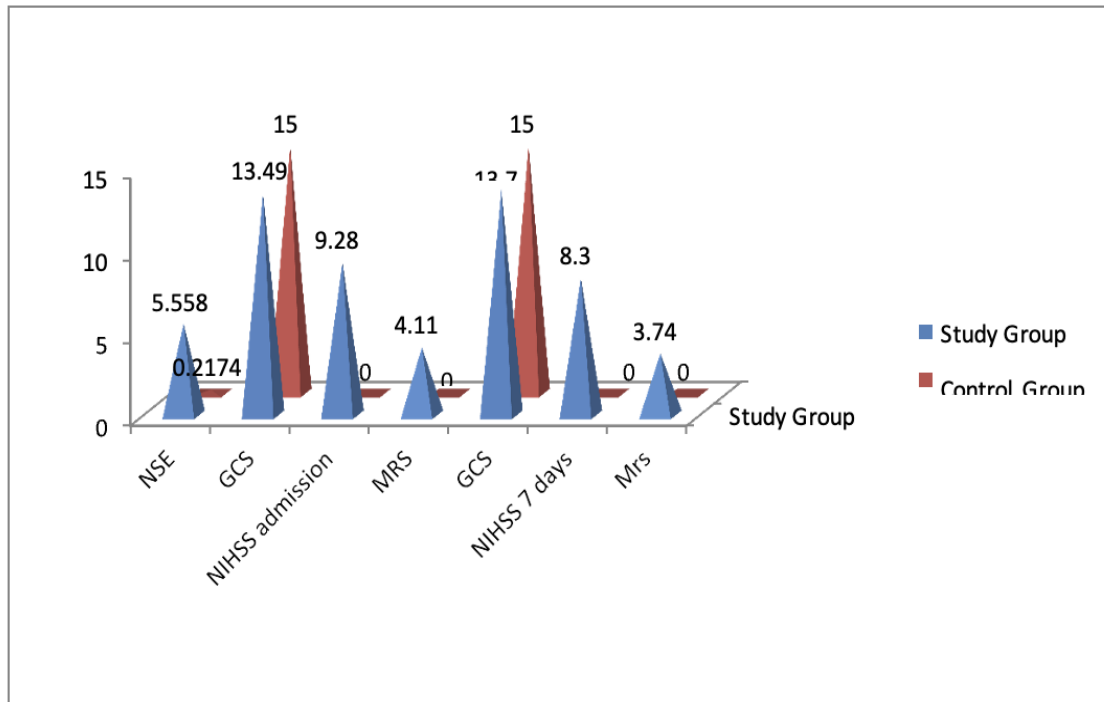
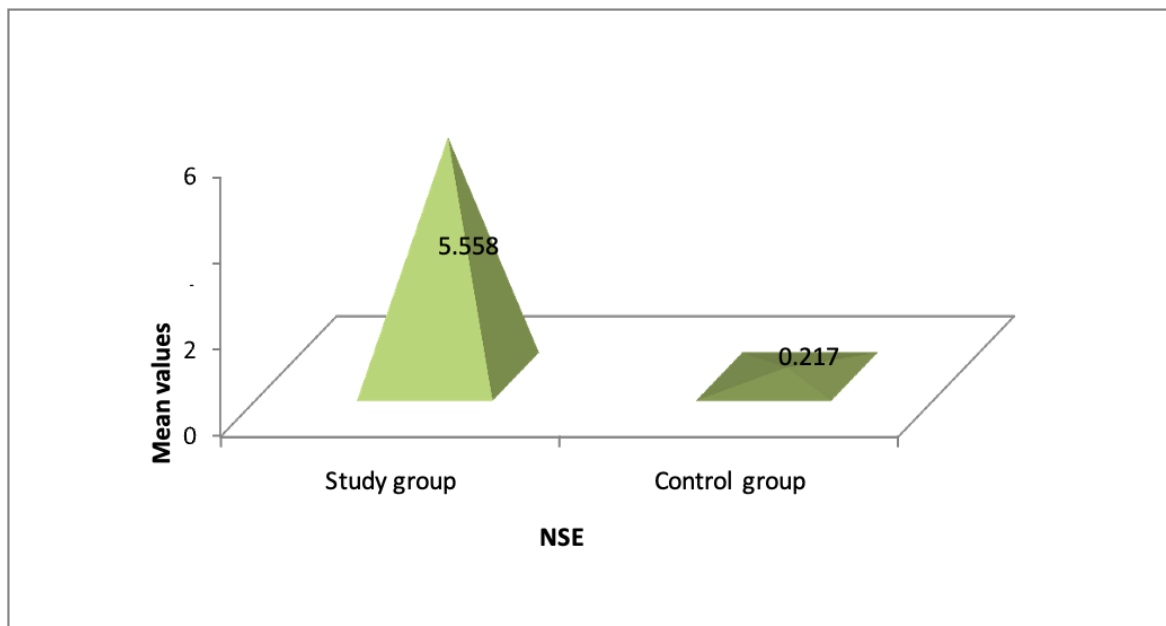


Table 3: Comparison of NSE Between Study and Control Groups

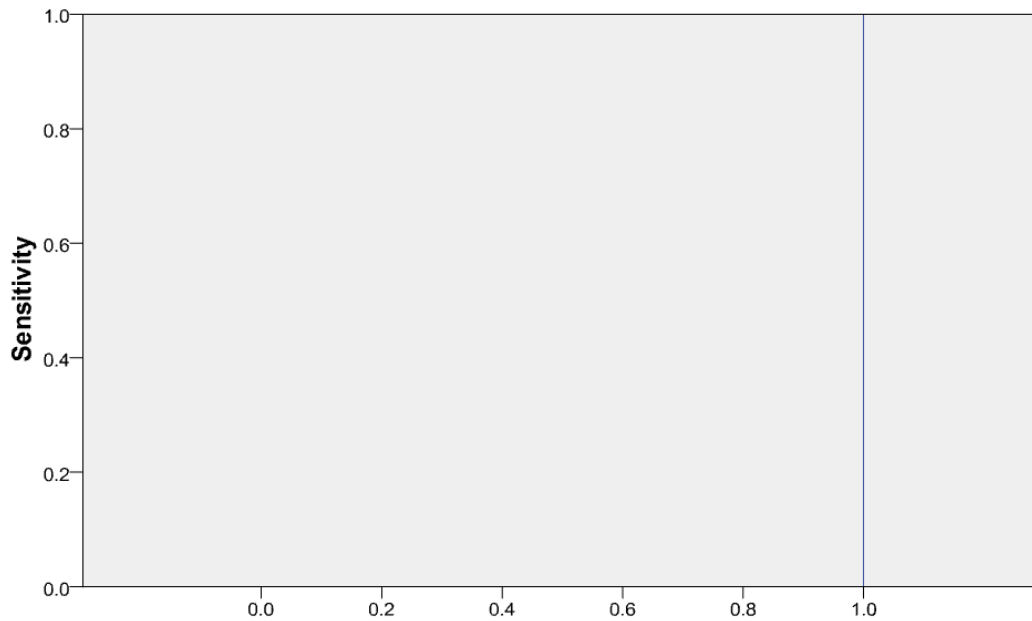
Comparison of	Study Group		Control group		Mann Whitney U test	P value	Remark
	Mean(Median)	±SD	Mean	±SD			
NSE	5.558(3.69)	5.049	0.217 (0.01)	0.516	U=76.5	P<0.0001	HS

HS: Highly Significant



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

ROC Curve



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

NSE	Study group	Control group	square test	P value
<1.48	5	45	X ² =68.365	P<0.0001*
≥1.48	42	02		
*: Highly significant				

Table 4:

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

Table 5: 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				

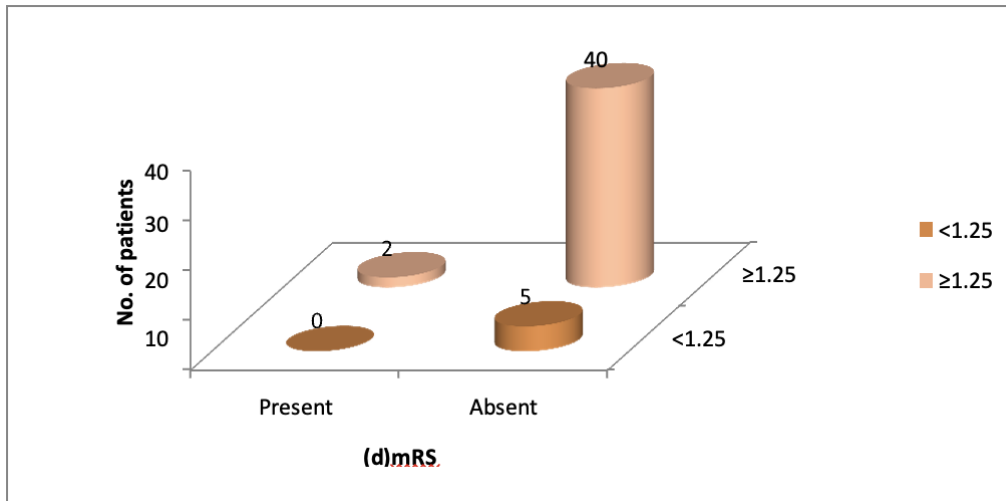
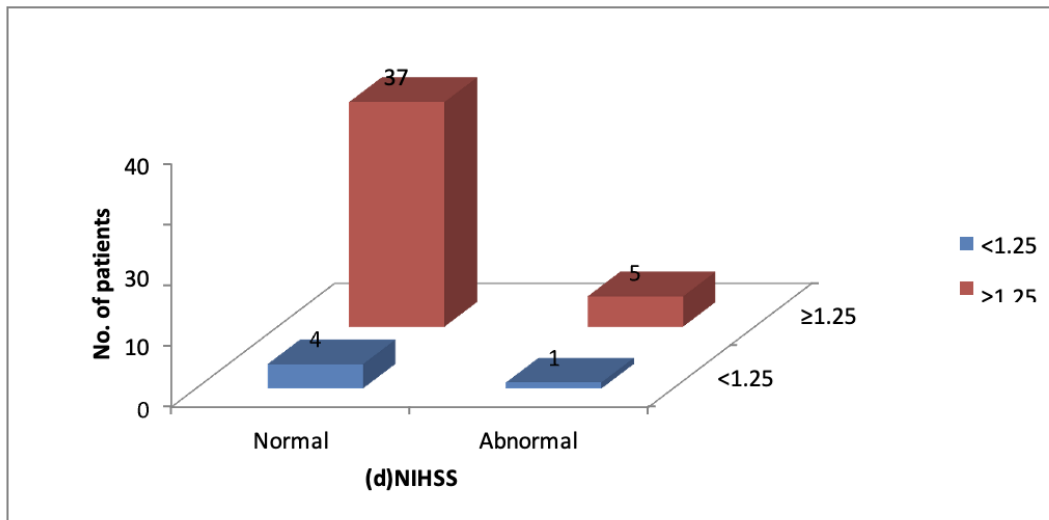


Table 6: Association between NSE and (d)NIHSS

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

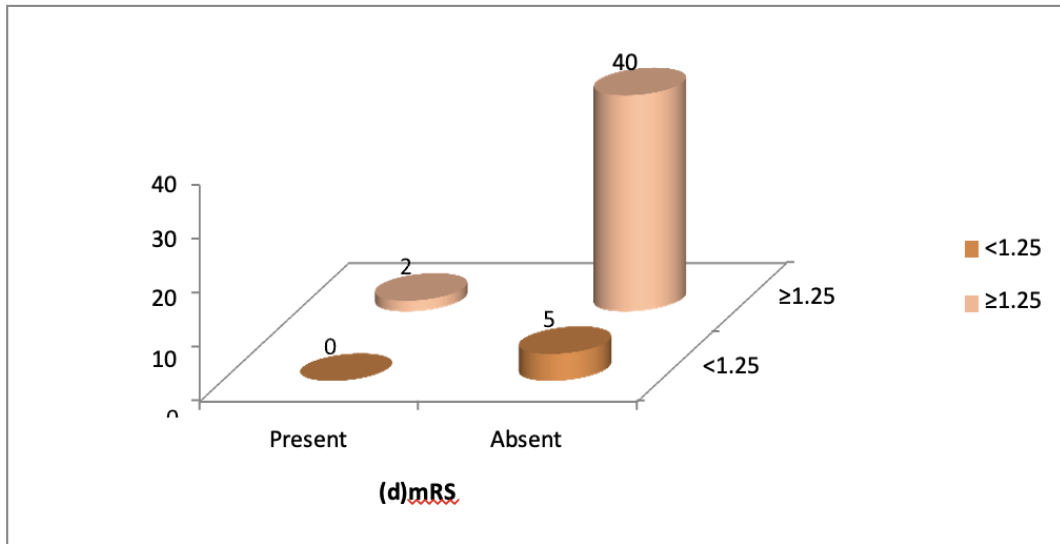


Highly significant statistical association exists between NSE and (d)NIHSS.

Table 7: Association between NSE and (d) mRS.

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

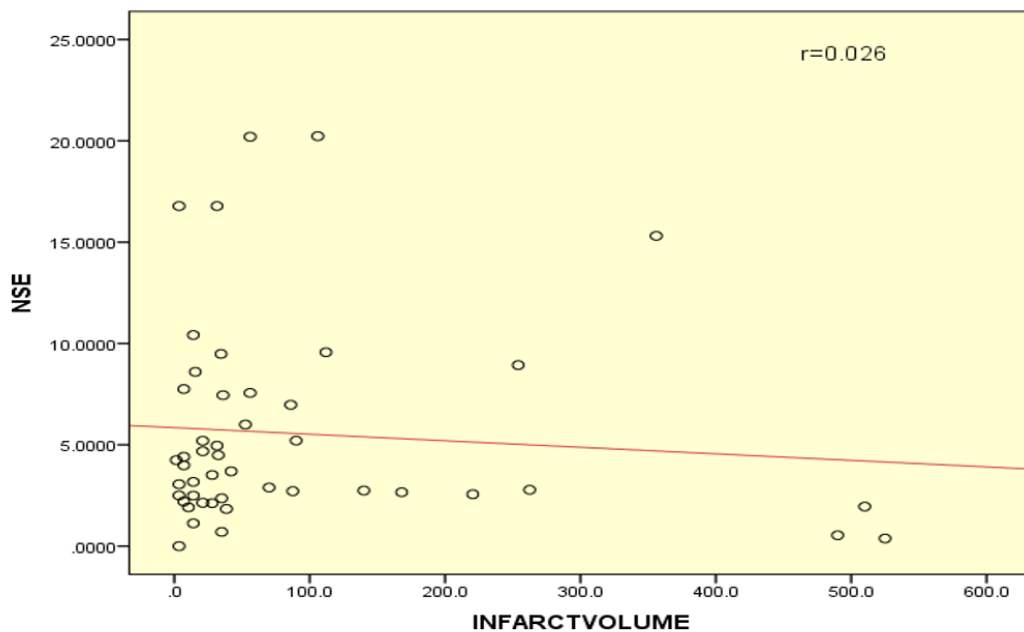
HS: Highly significant



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

TABLE 8: Correlation Between NSE and Infarct Volume

Correlation between	Spearman's Correlation coefficient	Interpretation	P value	Remark
NSE and Infarct 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 $=0.8642$.

Discussion

Acute ischaemic infarction is medical crisis that endangers sufferer'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 neurobiochemical markers having standard role in the diagnosis and treatment of acute infarction like NSE a neuronal form of the intracytoplasmic Glycolytic enzyme enolase. Various researchs 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 compared 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 al²(2012), Padalkar Ramchandra K et al³ (2014).

SERUM NSE	Anuradha bharosay et al ²	Padalkari 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. Raised 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 sufficiently sensitive marker of neural impairment which can be estimated in blood rather than in cerebrospinal fluid as blood samples taken in quick succession & independent of raised intracranial pressure compared to cerebrospinal samples.

Diagnostic performance of NSE	Padalakar ramchandra K et al ³	Naheer H Rawi 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, Naheer 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 accuracy (90%) $\geq 13.1 \mu\text{g/L}$. This cut-off threshold had maximum specificity (100%) & acceptable sensitivity (85%)⁴. Padalakariramchandra Kiet 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 0.84³

CORRELATION BETWEEN SERUM NSE AND GCS

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 quantifying infarct seriousness, however at latest few works utilized GCS for assessing infarct seriousness & clinical after effect. Work done by González García et al no compelling association found between GCS and infarct seriousness at presentation². 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 insignificant which is consistent with Missler et al, could not discovered compelling association among NSE levels & functional neurological outcome using GCS⁶.

CORRELATION BETWEEN SERUM NSE AND NIHSS (degree of disability/severity of stroke)

Correlation between NSE and (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 disability (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 NSE levels and neurological outcome measure⁴. Oh et al., predicted short term prognosis using NIHSS score at day 7 and found a significant correlation between initial NSE levels and NIHSS score on day 7 ($P<0.001$)⁷. Similar results obtained 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

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 their study assessed functional neurological outcome using mRS at 3 months, they also reported that patients with poor functional outcome (mRS >2) had significantly greater serum concentration of NSE ($P < 0.0001$) in cases of ischemic stroke, on multivariate analysis NSE at 72 h independently associated with poor outcome in this study also⁹. 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 are consistent with previous studies done¹⁰.

CORRELATION BETWEEN NSE AND INFARCT VOLUME

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 72 hrs 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 between initial serum NSE levels and infarct volume determined by T2 weighted MRI scan ($r = 0.81$)⁷. Zaheer *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 determining infarct volume.

Conclusion

Serum NSE levels were significantly high in cases compared to controls. Serum NSE levels were absolute significant sensitive marker for diagnosis of Acute ischaemic stroke patients within 72 hrs of onset of symptoms. Serum levels of NSE within 72 hrs of onset of symptoms in acute ischaemic stroke can be of use of foreseeing severity of stroke, degree of disability & initial functional neurological after effect. 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 stroke patients in the settings where 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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