Study Of Neuron-Specific Enolase As Potential Biomarker For Assessing The Severityand Outcome In Patients With Acute Ischaemic Stroke

by

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LIST OF ABBREVIATIONS

CVA	CEREBROVASCULAR ACCIDENT
NSE	NEURON SPECIFIC ENOLASE
GCS	GLASGOW COMA SCALE
NIHSS	NATIONAL INSTITUTES OF HEALTH STROKE SCALE
mRS	MODIFIED RANKIN SCALE
ROC	RECEIVER OPERATING CHARACTERISTIC
PVALUE	STATISTICALLY SIGNIFICANT

ABSTRACT

BACKGROUND:

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".

METHODOLOGY:

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.

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 off value 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 of acute ischaemic stroke p atients in the settings were CT scan,MRI scan not available or patients in whom CT sca n or MRI scan 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.

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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 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 Cerebrospinalfluid.

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.
0-NO STROKE,1-4-MINOR STROKE,5-15-MODERATE STROKE,16-20-MODERATE TO SEVERE STROKE,20-42-SEVERE STROKE.

Modified Rankin Scale (**mRS**), utmostly utilized clinical aftereffect magnitude for ischaemic infarct clinically. In patients endured agony of stroke, this scale consistently used to detect dependence in daily activities.

0 - None complaints.1 - None serious unability can do routine work, with few complaints.2 –little unability. can do self care needing no help, however can’t do

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.

AIMS AND OBJECTIVES

1. Figuring out significance of estimating NEURON SPECIFIC ENOLASE as potential brain biomarker for diagnosis, grade of weakness, neurodeficit deterioration as noticed succeeding 7days in predicting neurobhehavioural outcome, assessing the severity and prognostic parameter in acute ischaemic stroke.
2. To correlate NEURON SPECIFIC ENOLASE with infarct volume on 1st CT scan or and MRI brain imaging within 72hrs of onset of symptoms.

REVIEW OF LITERATURE

Ischaemic stroke perhaps as old as mankind. The earliest allusion of stroke like illness by apoplexia more than 2,400years ago by greek physician HIPPOCRATES [1,2].

Greek physician couldnot explain the situation truly occurs in head but to stasis of circulation, followed by every movement and activity of soul eliminated, and movement halted.

Galen 129AD goes for and evolves concept of greek physician, that occlusion engendered by something that halts movements of essential soul to head. His concept lasts for hundered of years.

1620-1695- European county medical specialist Johann Jakob Wepfer findouts that anything interrupts the circulation in the head of population expired of occlusion. In few deaths,significant haemorrhage in head. In others, the vessels blocked[1].

In 1928- Apoplexy is split into types according to etiology of the circulatory system, add to ennoble cerebral vascular accident (CVA) or ischaemic stroke[1].

In 1974, The Glasgow Coma Scale is developed to use by critical healthcare activity to cerebrovascular accident or brain trauma sufferers [3].

D schmechel et al (1978), NSE and NNE may be useful as specific metabolic markers for neurons and glial cells, respectively

In 1980-1985,NATIONAL INSTITUTES OF HEALTH STROKE SCALE made as investigation aid permitting steady stating of neural deficiencies in abrupt cerebrovascular trials, specifically initial studies of clot lysis [4].

A observation by Hans-Göran Hårdemark, M.D et al (1989),showed use of CSF markers like neuron specific enolase for evaluation of the extent of brain damage in experimental models and assist in understanding of their kinetics, which is important for use in clinical practice [5].

A study by R. T. CUNNINGHAM I. S. YOUNG et al (1991) showed blood neuron specific enolase can helpful indicator of neuronal damage in the stroke study,principally in assessment of treatment[6].

A study by Frank c Barone et al (1993) showed neurological dysfunction and infarction is associated with neuronal depletion and vascular redistribution of brain NSE leading to measurable increase in serum NSE[7].

Further shows diffusion of NSE from ischaemic tissues into the cerebral vasculature and systemic circulation to serve as a marker for the incidence of cerebral damage in acute and chronic ischemic brain infarcts[7].

Schaarschmidt H et al(1994),showed The unfolding regarding blood Neuronspecific enolase values used for the reason that significant measure for estimating prediction about brain infarction[8].

Schaarschmidt H further demonstrated as effective means to regulate place-filling ischaemic lesions & intracranial bleeds & add for enhancing treatment for serious ischaemic infarct[8].

Li Y et al (1995), research supports the view that CSF NSE can be a useful biochemical marker for brain ischemia[9].

Fang D et al(1995), research supports the view that CSF NSE can be a useful biochemical marker for brain ischemia[9]

RJ Butterworth et al(1996),showed estimation of NSE in the evaluating of early onset cerebrovascular accident patient with regard to analysis and anticipation of neurological aftereffect.

A study by R. T. CUNNINGHAM et al(1996),showed blood Neuron specific enolase is helpful indicator of infarct volume in studies of therapy in acute stroke[10].

Missler (1997) found intense association between cerebrovascular accident and infarct volume[11].

Sulter G et al(1998), Hyperglycaemia (blood glucose concentration > 7 mmol/l) complicating cortical ischaemic stroke found significantly higher NSE levels than in normoglycemic patients. NSE levels were not significantly different between normoglycemic and hyperglycemic patients of lacunar strokes[12].

A Study by Stevens H et al(1999),showed Serum N-acetyl-aspartate appears to be an early peripheral marker of ischaemically affected brain neurones, and the ratio of N-acetyl-aspartate to a protein marker, such as NSE, may serve as an index of irreversibility[13].

Oh SH et al(2002), study shows that early blood NSE value valuable parameter for intensity in ischaemic cerebrovascular accident, and associated with early and late clincial aftereffect[14].

Oh SH et al(2003), The initial serum NSE level is a reliable predictor for the extent of neuronal damage and the severity of clinical neurological deficits in acute anterior-circulation infarction[15].

A study by Selaković V et al(2003),showed Neuron-specific enolase concentration in cerebrospinal fluid and plasma may be an indicator of pathophysiological processes in the acute phase of brain ischemia and is significant in early diagnostics and therapy of the disease[16].

Tiainen (2003) found that blood NSE value in ischaemic infarct is a finer prognostic marker of neural injury contrast to Protein S 100B[17].

A observation by Yc wu et al(2004),showed blood nse values after acute stroke valuable for prognosis of lesion area and early- or late-clinical measure[18].

Anand N et al(2005),showed blood NSE value more in infarct sufferers contrast to non sufferers, and associated with infarct size[19].

Further shows, no association with clinical measure, and its correlation to cerebral infarct intensity is not clear.clarified due to difference in testing moments as NSE value elevate later,more than 24hrs[19].

Dwi Lily Lukas et al (2007)study shows blood NSE value in early onset infarct patients (24-48 hours) after infarct valuable marker to measure neuronal injury[20].

Al-Rawi NH et al(2009),Neuron specific enolase in saliva may utilized being important analytic & feasibly predictive means to estimate cerebral injury of sufferrers of infarct and infarct-associated disorders[21].

Guoping Tian et al (2009) The plasma level of NSE is greater among sufferrers of acute cerebral infarction compared to healthy cohort.it can reflect infarct severity and predict early prognosis of acute cerebral infarction[22].

Natheer H Rawi and Karim M Atiyah (2009) acheived analytical effeciency of Neuron specific enolase following cerebral infarction.As reported, the region beneath the receiver operating characteristic curvature of blood neuron specific enolase considerably more contrast to neuron specific enolase in saliva .The maximum level for blood NSE revealing the uppermost analytical precision (90%) ≥ 13.1μg/L.maximum limit revealed ideal uniqueness (100%) and insight awareness (85%)[21].

González-García S et al (2012), The proportions of serum levels of NSE and S100B after early onset

Ischaemic infarct medically appropriate to anticipate clinical aftereffect and post-infarct despair[23].

A study by Bharosay A et al(2012) showed The maximum serum NSE level within 72 h of admission was significantly higher in patients with greater degree of disability at the time of admission[24].Blood NSE has great prognostic importance for assessing intensity and initial clinical aftereffect abrupt ischaemic infarct[24].

Singh HV et al(2013), blood levels of NSE and IL-10 have great prognostic importance for initial clinical aftereffect of abrupt ischaemic infarct[25].

Aparna Pandey et al (2013) early blood NSE values valuable for intensity in acute cerebral infarction and associated with functional weakness and early clinical aftereffect[25].

Zaheer S et al (2013)blood nse value initial week of cerebral infarction used to anticipate neurological weakness and initial clinical aftereffect[26].

Pandey et al(2014), The highest blood NSE and CRP values during 72 h of stroke considerably greater in sufferers with more grade of disablement at the time of stroke. Both serum markers appreceably associated with clinical weakness and early aftereffect[27].Observation showed that blood markers NSE and CRP have great prognostic importance for assessing intensity and initial clinical aftereffect ischaemic infarct[27].

Kim BJ et al (2014) Serial NSE analysis throughout abrupt duration for cerebral infarction, useful for monitoring hemorrhagic transformation and the blood-brain barrier disruption status[28]

Padalkar Ramchandra K et al (2014) showed,blood neuron specific enolase is cerebral serum marker of ischaemic stroke was increased contrast to normal cohort leads to understanding around pathogenesis in cerebral infarction[29].Investigatiors found blood neuron specific enolase of stroke sufferrers following inception (<72hrs) useful for evaluating proportions of brain injury & dependable measure to scale ischaemic stroke[29].

Haupt WF et al (2016), Predictive level of somatosensory evoked potentials, nse, and S100 for short-term outcome in ischemic stroke complement each other[30].

ACUTE ISCHAEMIC STROKE

The word stroke is based on abrupt localized neural disorder, appropriately the varitey because of brain circulatory disorders. The word cerebrovascular disease defines all pathology of the neurology due to abnormal physiology of the circulatory system.

“Pathologic mechanism includes—namely, blokage of the vessel by embolus or thrombus, rupture of a vessel, an impaired permeability of the vessel wall, or increased viscosity or other change in the quality of the blood flowing through the cerebral vessels.”

Stroke is defined rapidly developing clinical symptoms and signs of focal, and at times global, loss of cerebral function lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin.

Ischaemic stroke is defined as stroke due to vascular insufficiency (such as cerebrovascular thromboembolism) rather than haemorrhage.

Ischaemic stroke manifest within seconds because neurons lack glycogen,so energy loss is rapid,may present symptoms of sudden onsent of any of the following loss of sensory and or motor function on one side of body (nearly 85% ischaemic stroke patients have hemiparesis),change in vision,gait,or ability of speak or understand or a sudden severe headache.

CLASSIFICATION

The etiology of ischemic stroke affects prognosis, outcome, and management.Various stroke etiologic classification systems have been developed.

The TOAST classification system is the most commonly used in patients with ischemic stroke.

"(TOAST) Trial of Org 10172 in Acute Stroke Treatment[31]

A classification of subtypes devised using clinical features and the results of ancillary diagnostic studies.

The TOAST classification - five subtypes of ischemic stroke:

1) Major-artery atherosclerosis- Important brain artery or branch cortical artery >50% stenosis or occlusion,due to atherosclerosis(cortical or cerebellar dysfunction,no lacunar syndrome, cortical, cerebellar, brain stem or subcortical infarct >1.5 cm; stenosisof extracranial internal carotid artery;no other abnormalities on tests),no cardiac source of embolism;– no subcortical or brainstem infarct <1.5 cm.2

2)cardioembolism-

a)high-risk

[mechanical prosthetic valve, mitral stenosis with AF, AF other than lone AF, left atrial/atrial appendage thrombus, sick sinus syndrome,recent myocardial infarction (<4 weeks), leftventricular thrombus, dilated cardiomy-opathy, akinetic left ventricular segment, atrial myxoma, infective endocarditis]

b)Medium risk

[mitral valve prolapse,mitral annulus calcification, mitral steno-sis without AF, left atrial turbulence (smoke), atrial septal aneurysm, patentforamen ovale, atrial flutter,lone AF, bioprosthetic cardiac valve, nonbacterial thrombotic endocarditis, congestive heart failure, hypokinetic left ventricular seg-ment, myocardial infarction >4 weeks and <6 months]

3) Small-vessel occlusion, one of the traditional clinical lacunar syndromes and no evidence of cerebral cortical dysfunction

a. A history of diabetes or hypertension supports the diagnosis

b. Should have also a normal CT/MRIexamination or a relevant brain stem or subcortical hemispheric lesion with a diameter of <1.5 cms.

c.Should not fulfill criteria for large-artery atherosclerosis or cardioembolism.

4) Stroke of other determined etiology, nonatherosclerotic vasculopathies, hypercoagulable states, hematologicdisorders;– cardiac source of embolism or large-artery atherosclerosis should be excluded.

5) stroke of undetermined etiology. two or more causes identified;– negative evaluation;– incomplete evaluation."

Utilizing TOAST classification,between clinicans acceptance is good.TOAST classification uncomplicated & with very high among clinicans accord. This classification incur clinicans delineate reciprocation of management between paramount class of sufferrers of cerebral infarct.

Fragility of this system of classification-

"1.Small M1vessel (lacunar) stroke is defined by the clinical syndrome and the size of the infarct. Consequently, a single small deep infarct due to M1 middle cerebral artery atherosclerotic stenosis could be classified by researchers as small vessel disease if an appropriate M1 middle cerebral artery evaluation has not been performed.2. Embolism from cardiac source are divided as high or medium risk.A patient with dissection of V3 vertebral artery uncared by incomplete work-up and patent foramen ovale (PFO) can be identified by clinical investigator as a 'cardiac embolism[31]."

Large number physicians to excess estimate the 'infarction of unknown cause'group into sufferers with minor lumen disorder.

Other system of classification recently developed-

ASCO (A for atherosclerosis; S for small-vessel disease, C for cardiac pathology, and O for other causes) CLASSIFICATION OF STROKE. The A-S-C-O classification system takes into account combination of mechanism doesnot determine final stroke etiology,this approach more descriptive.

RISK FACTORS

Non-modifiable risk factors

Age,gender,race,ethinicity,heredity.

Modifiable risk factors

Hypertension,atrial fibrillation,cardiac diseases, hyperlipidemia, diabetes mellitus, cigarette smoking,physical inactivity, carotid stenosis, alcoholism, transient ischaemic attack. Antiphospolipid syndrome, elevated homocysteine, chronic infection like Chlamydia pneumoniae, periodontal disease.

Age-elderly >80yrs of age

Race-blacks>Hispanics>whites

Sex-men>women,except in young (35-44yrs)

Heredity-monozygotic twins,dominant genetic disorders CADASIL

HYPERTENSION

"Most significant modifiable risk factor of stroke.Arterial hypertension shares upto 60% of all strokes by pathogenesis like an atheroma in carotid,vertebral arteries and aortic arch and friability of small cerebral arteries,left ventricular dysfunction,atrial fibrillation.Treatment if bp >140/90mmhg.Metaanalysis of randomized control trials confirms reduction in stroke about 30-40% with blood pressure lowering [32]."

DIABETES MELLITUS

Dangerous element for earliest ischaemic infarct not for repitative ischaemic infarct.Diabetic stroke patients have more severe disability,slower rate of recovery,higher rates of stroke recurrence and higher mortality than non-diabetic stroke patients.

TOBACO AND CIGARETTE SMOKING

Active and passive smoking constituted separate dngerous element for foremost ischaemic infarct,doubled danger of infarct compared to non-smokers.risk of stroke with smoking seen in all ages,both sexes and all race/ethnic groups.Pathogenesis involved in increased risk due to changes in hemodynamics and stenosis of vessels.

ALCOHOL CONSUMPTION

Large amount liquor correlated with increased rates of ischaemic infarct recurrence. Mechanism involved are "Alcohol induced hypertension, hypercoaguable states, reduced cerebral blood flow and atrial fibrillation[32]".

COMMON CAUSES OF STROKES IN YOUNG (<40yrs)

Cerebral embolism(rheumatic heart disease).

Arteritis or vaculitis (tuberculosis,syphilis,takayasu's disease,collagen vascular disease) Hyperviscosity syndrome (polycythemia,thrombocytosis) demyelinating isease(multiple sclerosis) Encephalitis or meningoencephalitis.

Anticoagulant therapy

Cerebral abscess

Hemiplegic migraine

Venous sinus thrombosis-puerperium

Drug abuse-amphetamine or cocaine

Cerebral malaria

hysteria

ETIOLOGY OF CRYPTOGENIC STROKE

CARDIOVASCULAR

Aseptic thrombus,infective endocarditis,left atrial myoxma, transient or persistent atrial fibrillation-especially with left atrial enlargement and aged >65yrs,LV thrombus, aortic or carotid atherosclerosis, valvular heart diseases(mitral valve prolapsed, mitral annular calcification, calcific aortic stenosis,prosthetic valve replacement).

NON-CARDIOVASCULAR

Hypercoaguable states(protein c or s deficiency, factor v leiden mutation, prothrombin G20210A mutation, anti-thrombin deficiency, anti-phospholipid antibodies)

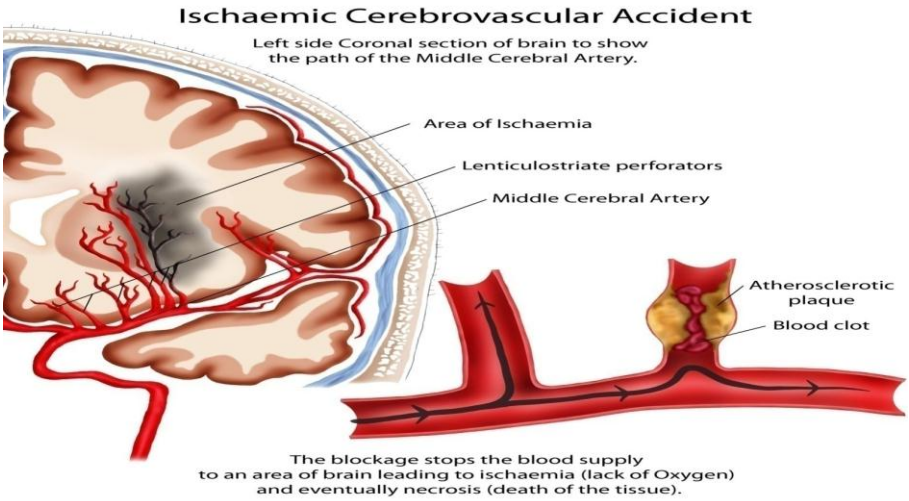
ATRIAL FIBRILLATION

Most quotidian cardiac cause for stroke,45% contribution of all embolic ischemic strokes of cardiac cause.Main mechanism of stroke in AF is a embolus originating from a left atrial thrombus.Patients with AF and prior stroke or transient ischemic attach is estimated risk 12% per year.Patients with spontaneous by transesophagial echo contrast or thrombus in the left atrium had a risk of recurrent stroke of 7.5%.

AF only reasons to use anticoagulation for secondary stroke Prevention.For almost all other reasons antiplatelet agents are used.

HYPERCOAGULABILITY STATES

Hypercoagulability states in patients under 55 years with ischemic stroke are a very common finding,affecting up to 46%.Hyperhomocysteinemia, with or without concomitant mutation is the most frequent abnormality followed by Leiden factor V mutation.



PATHOPHYSIOLOGY

"Brain clotformation- relates to the devlopment of a circulatory coagulam within vessel like internal carotid artery,proximal and intracranial vertebral arteries develop into lacunes, minor infarcts to characteristic areas like basal ganglia, thalamus, internal capsule, pons and cerebellum that forms at the blocked area of the artery [32]".

Arteriosclerosis leads to vessel blockage forming into ischaemic infarct.Atherosclerotic plaques go through abnormal modifications such as block,alteration of endothelium in this modification triggers a intricate changes that stimulate different catastrophic vessel effective catalysts.

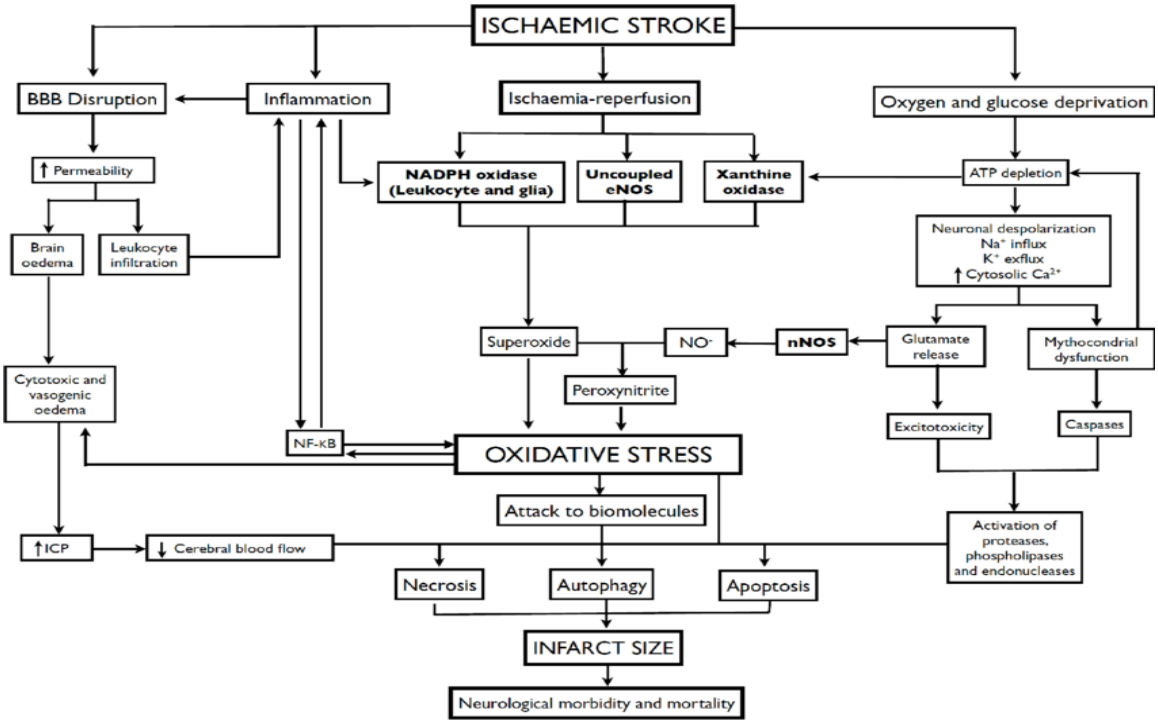
Platelet attachment & collection on vessel lumen,causes minor foci of platelets and fibrin.clot formation in the extracranial and intracranial arteries occurs due to roughening of intima and plaque formation,on this plaque platelets attach and collect, then activation of coagulation and clot forms at area of plaque. When the redressal process of collateral blood supply unsuccessful, interomission is jeopradized, causing necrosis.Extracranial vessel narrowing inclined to impairement and plaque break causing to brain thromboembolism. Thromboembolic block of main or many minor intracerebral vessels leads to specific area decrease of circulation causing to additional formation of clot in microvasculature.

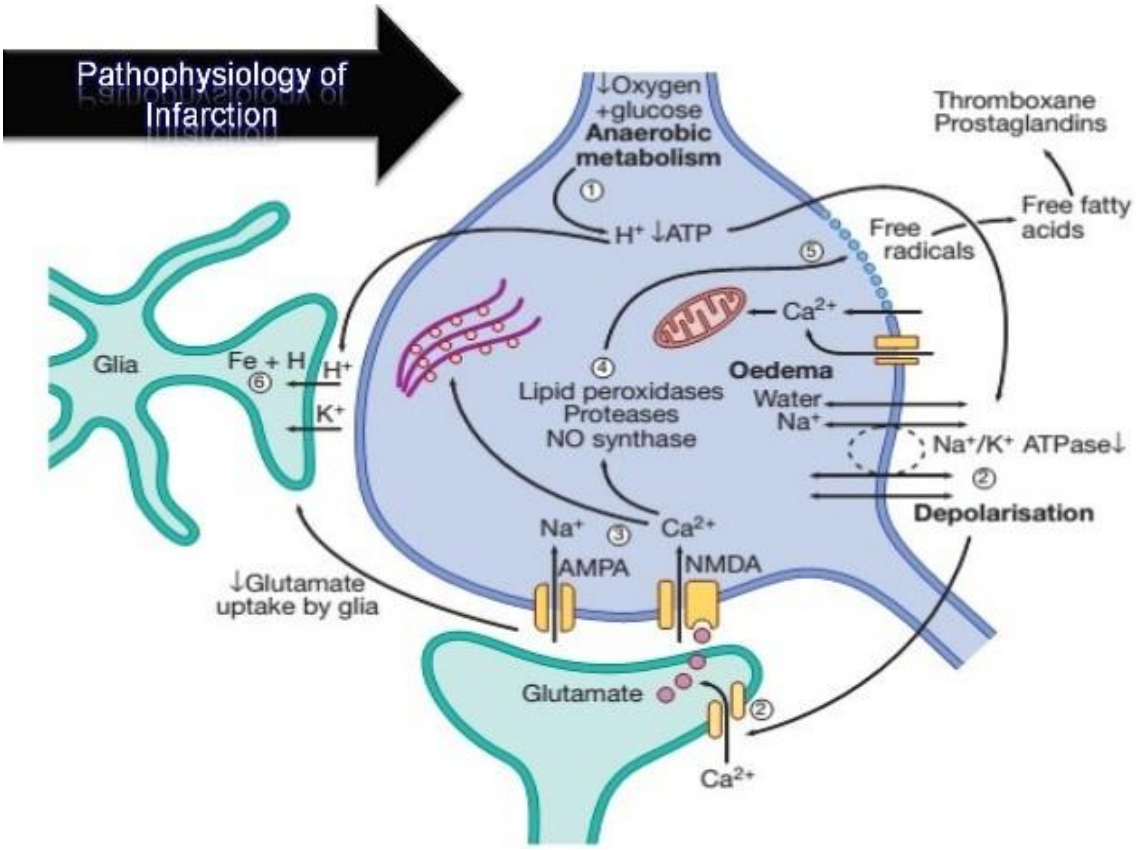
EMBOLISM- “Cerebral embolism means a circulatory coagulum developed at separate area in the vascular system,such as heart and main arteries of the upper chest and neck.ischaemic infarct deveops when coagulum ruptures, driven by the blood flow and stucked in medium sized ramifiying vessels.Microemboli rupture from a sclerosed plaque in the carotid artery or from cardiac sources such as atrial fibrillation,or a hypokinetic left ventricle.Commonly identified cardiac sources for embolism include atrial fibrillation,sinoatrial disorder,recent acute myocardial infarction (AMI), subacute bacterial endocarditis, cardiac tumors,and valvular disorders,both native and artificial[32]”.

Somewhere around one third of cerebral infarction, clot to cerebral structure initiates from cardia,particularly in atrial fibrillation.other than coagulam, fibrin,chunks of atheromatous plaque,components embolize into the brain blood supply like “fat,air,tumor or metastasis,bacterial clumps,and foreign bodies add to this process[32]”. Based on cerebovascular disease directories of developed nations, cardioembolism utmost typical etiology leading to cerebral infarction.Embolic infarction generally manifest with neurodeficit that is highest at inception.

GLOBAL ISCHAEMIA OR HYPOTENSIVE STROKE

“Global reduced perfusion due to systemic reduction of blood pressure.Many mechanism cause global hypoperfusion,the most common understood is cardiac arrest because of myocardial infarction and/or arrhythmia or hypotensive shock.The pyramidal cell layer of the hippocampus and the Purkinjecell layer of the cerebellar cortex areas are mainly involved[32]”.Universal reduction of blood supply is bad because there will be buildup of lactic acid and different poisonous metabolities,usually cleared by blood in extra to energy breakdown contrast to hypoglycaemia,hypoxia and seizures.abrupt hypotension due to extracranial activities like “heart failure, occult haemorrhage, or multiple pulmonary emboli contribute to critical infarcts in old aged sufferers[32]”.


ISCHAEMIC STROKE
BBB Disruption
Inflammation
Ischaemia-reperfusion
Oxygen and glucose deprivation
↑ Permeability
NADPH oxidase (Leukocyte and glia)
Uncoupled eNOS
Xanthine oxidase
ATP depletion
Brain oedema
Leukocyte infiltration
Neuronal despolarization
Na+ influx
K+ efflux
↑ Cytosolic Ca2+
Superoxide
NO·
nNOS
Glutamate release
Mythocondrial dysfunction
Cytotoxic and vasogenic oedema
Peroxynitrite
Excitotoxicity
Caspases
NF-kB
OXIDATIVE STRESS
Attack to biomolecules
↑ICP
↓ Cerebral blood flow
Activation of proteases, phospholipases and endonucleases
Necrosis
Autophagy
Apoptosis
INFARCT SIZE
Neurological morbidity and mortality


Pathophysiology of Infarction
↓Oxygen
+glucose
Anaerobic metabolism
Thromboxane
Prostaglandins
Free fatty acids
H+ ↓ATP
Free radicals
Ca2+
Glia
Fe + H
H+
K+
Oedema
Lipid peroxidases
Proteases
NO synthase
Water
Na+
Na+/K+ ATPase↓
Depolarisation
Na+
Ca2+
AMPA
NMDA
↓Glutamate uptake by glia
Glutamate
Ca2+

In fraction of seconds following reduction in circulation for cerebral area, ischemic cascade swiftly beguns.because of interruption of circulation to the region causes restriction of the transport of oxygen and metabolic elements to nerve cells leading to ATP decreasing and energy exhaustion.This constitutes a seauence of ensuing biochemical events that finally cause dissolution of cellular surface & neuronal cellular demise in ischaemic centre[32].

Biological activities comprise of: ionian disparity,discharge of excess glutamate into interstitial area causing excessive stimulation by neurotransmitters, a abrupt rise in calcium inside cell stimulating several intracellular destructive mechanism like mitochondrial dysfunction,bloodbrain fence malfunction,oxigenous & nitrosative strain & trigger following inadequate blood supply leading to swelling,eventually to demise of neurons, glia & endothelial cells.In the penumbra area around the infarct centre, tissue is conserved for a spcific time duration based on if circulation is rejuvenate[32].

"In general,neurons and oligodendrocytes seem to be more vulnerable to cell death than astroglial or endothelial cells,and among neurons, CA1 hippocampal pyramidal neurons, cortical projection neurons in layer 3, subsets of neurons in dorsolateral striatum and Purkinje cells of the cerebellum are particularly susceptible[32]".

Huge stores of substitute substance to sugar,like animal starch, lactatic acid and transfat, with regard to embden Meyerhof pathway and oxidation are existing within cerebrum, however o2 cannot be replaced within mitochondrial oxidative phosphorylation, principal provider for ATP within nerve cells[32].

Decreased ATP activates the glycolysis concering left over sugar & animal starch,leads to buildup of protons and lactate and so intracellular acidification.Outcome is reduction of ATP levels causing stoppage of the electron transport chain activity inside power house of cell and causing interrupting ionian pumps such as Na+-K+-ATPase.Ca2+-H+ATPase, reversal of Na+-Ca2+transporter causing rise within cell Na+, Ca2+, Cl density & removing potassium[32].

Reorganizing ionian crossing cellular surface depolarizing nerve cells & astrocytes,causing abundant discharge of neurotransmitters (glutamate) leads to nerve cells excitotoxicity[32].

“Huge amounts of intracellular calcium, ROS, and RNS cause cell death by:
1)Activate proteases so as to alter cellular configuration i.e. protein, DNA, lipid,
2)peroxident lipids,alters covering integrity.
3) stimulate microglia to discharge cytotoxic factors,
4)interrupting mitochondrial function, and
5)inducing pyknosis (chromatin condensation)[32]

“The opening of permeability transition pore results in mitochondrial depolarization,induction of calcium deregulation and induction of neuronal death by damaging dendrites and synaptic connections[32]”.

O2levels are exhausted before glucose in ischaemic cells,indulging a swap to the glycolytic pathway of anaerobic ATP generation.outcome is lactic acid and H+ generation inside mitochondria and the consequent reversal of the H+uniporter on the mitochondrial membrane leads to excess cytosolic H+ build up and acidosis[32].

Oxidative stress caused by acidosis by donating H+ for transformation of •O2−into H2O2 or OH-[32]

The restoration of blood supply after ischaemia causes generation of superoxide and hydroxyl radicals overcomes internal salvage pathways.Superoxide cause oxidative damage of iron/sulfur clusters of aconitase,an valuable enzyme in the tricarboxylic acid cycle[32].

Stimulation of nitric oxide synthase(NOS) when ischaemia going on cause abundant nitric oxide generation causing nitrosative damage by nitrosylation of protein heme sites (e.g. cytochrome c) and metabolites with oxygen or other nitrogen oxides.O2 interact with nitric oxide (NO) to generate peroxynitrite ONOO,powerful oxidative radical leads to protein nitration and malfunction[32].

“Lipids, proteins and DNA disrupted by hydroxyl radical,peroxynitrite and peroxynitrite metabolites (hydroxyl radical, carbonate radical and nitrogen dioxide. Glutamate activates NMDARs, increases intracellular NO and subsequent ONOO production in the ATP depleted post synaptic cell[32]”

SYMPTOMATOLOGY

Onset is less rapid than cerebral embolism or haemorrhage usually with stepwise progression. There may be transient loss of consciousness occurs during sleep or soon after arising from bed.

Spastic dysarthria -Difficulty in uttering words but pronounced clearly.

Hemiplegia or hemiparesis.Facial palsy.Hemianopia.

TRANSIENT ISCHAEMIC ATTACK

The American heart association/American stroke association defines TIA as a brief period of neurological dysfunction with a vascular cause with clinical symptoms typically lasting less than one hour and without evidence of infarction on imaging[33].

Symptoms include unilateral paresis,dysarthria, transient monocular blindness, hemianopia, Diplopia.

STROKE IN EVOLUTION- Patients with an evolving stroke may initially present with mild symptoms that seem to worsen upon re-examination, even if proper treatments have been initiated. Up to 24 to 72 hours (depending on the area of the brain affected) worsening of clinical signs.

COMPLETED STROKE-defined as neurologic deficit due to occlusive cerebrovascular disease which persists for hours or days.

CLINICAL FEATURES

1.ANTERIOR CEREBRAL ARTERY OCCLUSION

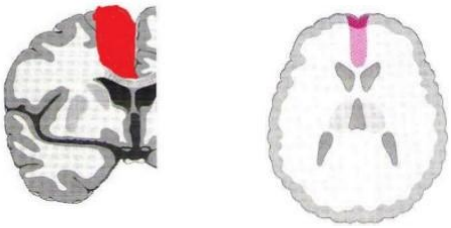
Contralateral

a. Paralysis of leg with paresis of arm

b. Cortical sensory loss over legs

Urinary incontinence

Gait apraxia, emotional disturbance, presence of relaxed reflexes.



2.MIDDLE CEREBRAL ARTERY OCCLUSION

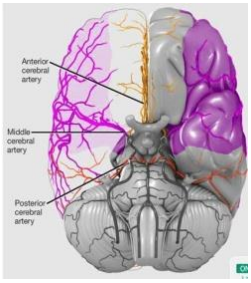
Contralateral hemiplegia

Contralateral hemisensory loss

Contralateral gaze palsy

Contralateral hemianopia,

global dysphasia,ansognosia



3.POSTERIOR CEREBRAL ARTERY OCCLUSION

CLAUDE'S SYNDROME-3rd nerve palsy

\+ → rednucleus/cerebral

Contralateral ataxia peduncle

WEBER'S SYNDROME-3rd nerve palsy+hemiplegia-mid brain

BENEDIKT'S SYNDROME-3rd nerve palsy+hemiplegia+ataxia

LATERAL MEDULLARY SYNDROME-

Contralateral hemisensory loss+agonizing pain.

LOCALIZATION IN HEMIPLEGIA

1.cortical infarction

Contralateral monoplegia

Jacksonian convulsions,headache at onset

Speech abnormality

Flexors and extensors of both upper and lower limb equally involved.Astereognosis

2.subcortical infarction or at level of corona radiata

Contralateral monoplegia with absence of cortical signs

Impairment of tacticle localization and discrimination in affected limbs

3.Internal capsule

Most common site of lesion in a CVA.Dense hemiplegia.

Complete hemiplegia on opposite side with only UMN type of 7^{th} cranial nerve involvement on side of paralysis

Hemianaesthesia, Homonymous hemianopia,global aphasia.

4.Brain stem infarction-Crossed hemiplegia 5.Spinal hemiplegia-ipsilateral hemiplegia.

WATERSHED STROKES

Watershed areas are at high risk of developing ischemia or lack of blood flow, during extreme drops of blood pressure. Common triggers for watershed strokes include periods of extreme dehydration, arrhythmias and sepsis

Hypoperfusion and extreme drops in blood pressure caused by cardia arrythmias like asystole, atrial fibrillation with slow ventricular response,non profusing ventricular tachycardia,ventricular fibrillation,high grade heart block etc.

LACUNAR INFARCTS-

Small, deep infarcts developed due to atherothrombotic or lipohyalinotic blocking perforating blood vessel(30-300microm) within brain substance. Major risk factor remains hypertension. Presents as-Pure motor hemiparesis, pure sensory stroke, Ataxic hemiparesis Clumsy hand, Dysathria syndrome.

DIAGNOSTIC CRITERIA FOR ACUTE ISCHAEMIC STROKE

In acute stroke imaging evaluation aimed at arriving diagnosis immediately and to get precise data about intracranial vasculature and brain perfusion for supervising specific therapy. Intraarterial thrombolysis is typically restricted to less than six hours. Exceptions to this general rule include basilar artery thrombosis and patients outside the six-hour window who have a persistent significant perfusion-diffusion mismatch.

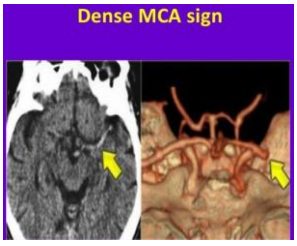
Generally accepted imaging criteria for intraarterial thrombolysis include involvement of less than one-third of the MCA territory and absence of parenchymal hemorrhage.

"BRAIN ATTACK" PROTOCOLS. The primary goals of emergent stroke imaging are (1) to distinguish "bland" or ischemic stroke from intracranial hemorrhage and (2) to select/triage patients for possible reperfusion therapies[34].

Most protocols begin with emergent NECT to answer the first "must know" question in stroke imaging: Is intracranial hemorrhage or a stroke "mimic" (such as subdural hematoma or neoplasm) present?.

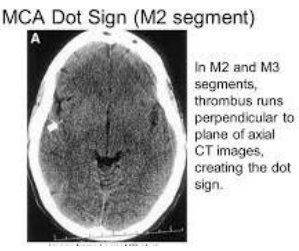
During the first hours after acute ischemic stroke, the CT does not usually show much in the first 24 hours[33].In first 6hrs normal CT brain **does not excludes the presence of ischemic stroke** because of low negative predictive value (27%)[35]

NON-CONTRAST ENHANCED CT SCAN



Early abnormal findings on CT scan have been described as

The most specific but least sensitive sign is a hyperattenuating vessel filled with acute thrombus . A "dense MCA" sign is seen in 30% of cases with documented M1 occlusion[34] .



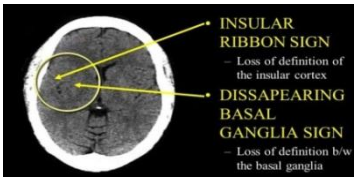
Less common sites for a hyper-dense vessel sign are the intracranial internal carotid artery , basilar artery , and MCA branches in the sylvian fissure ("dot" sign) .

Uncommon but important NECT findings that indicate vascular occlusion include a calcified embolus, most likely from an "at-

risk" ulcerated atherosclerotic plaque in the cervical or cavernous ICA

Blurring and indistinctness of gray-white matter (GM-WM) interfaces can be seen in 50-70% of cases within the first three hours following occlusion[34] .

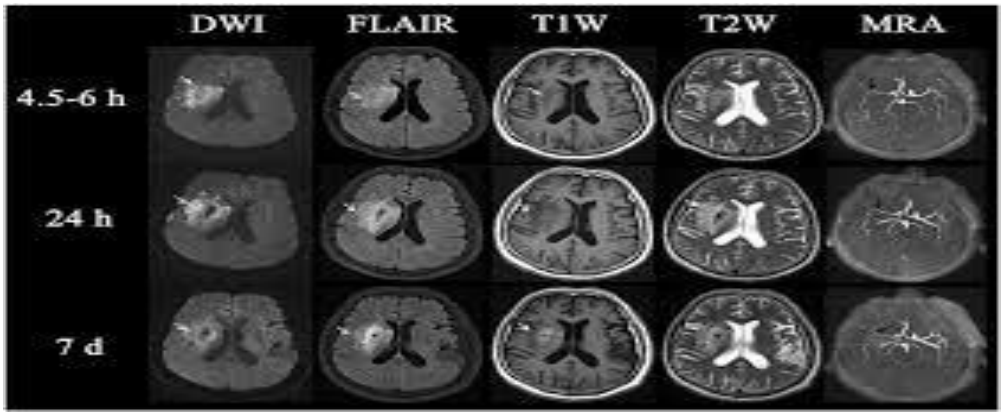
Loss of the insular cortex ("insular ribbon" sign) and decreased density of the basal ganglia ("disappearing basal ganglia" sign) are the most common findings[33].



MRI FINDINGS

T1WI.T1WI is usually normal within the first three to six hours. Subtle gyral swelling and hypointensity begin to develop within 12-24 hours and are seen as blurring of the GM-WM interfaces. With large vessel occlusions, loss of the expected "flow void" in the affected artery can erratically be distinguished.

T2/FLAIR. Only 30-50% of acute strokes show cortical swelling and hyperintensity on FLAIR scans within the first four hours. Nearly all strokes are FLAIR positive by seven hours following symptoms onset[34].

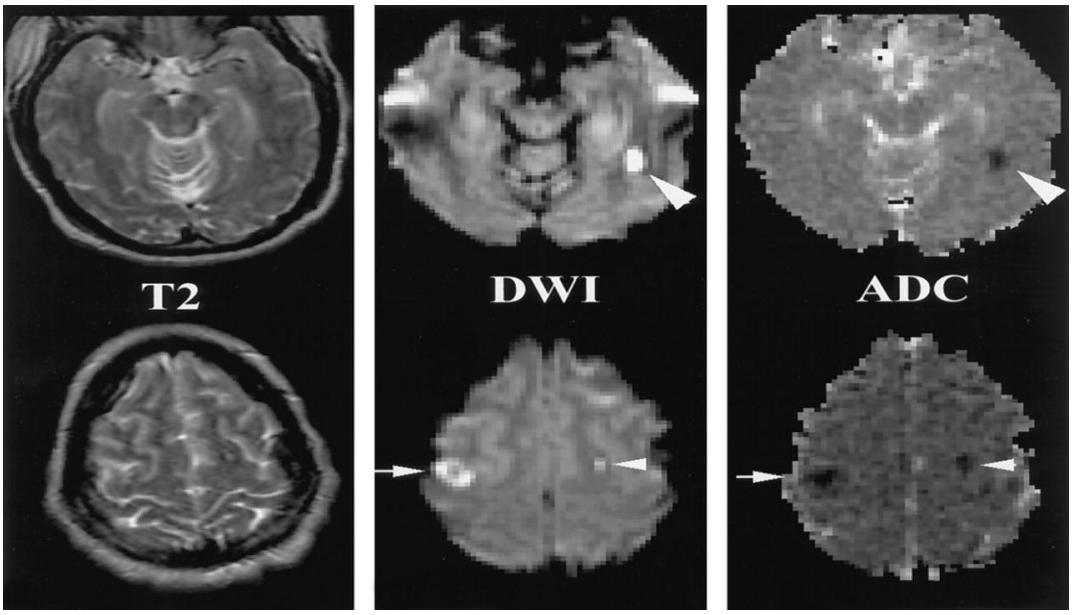


T2 scan becomes positive generally within 12-24hrs.

Intraarterial hyperintensity on FLAIR is an early sign of stroke and indicates slow flow (not thrombosis), either from delayed antegrade flow or—more commonly—retrograde collateral filling across the cortical watershed[34] .

FLAIR-DWI "mismatch"(negative FLAIR, positive DWI) has been suggested as a quick indicator of viable ischemic penumbra and eligibility for thrombolysis[34].

DWI and DTI. Cellular swelling begins to develop within minutes following an ischemic insult. ADC values decrease,producing high signal intensity on DWI images[34] .



Cytotoxic edema as the basis for decreased ADC, part of the decrease is due to reduced water diffusibility caused by decreased levels of astrocytic aquaporin-4 (AQP4).

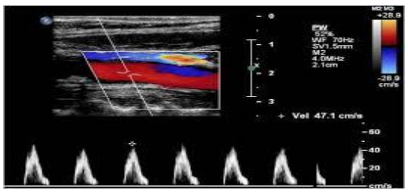
Aquaporins are transmembrane proteins—water channels—that facilitate bidirectional selective water transport in and out of the cell.

Around 95% of hyperacute infarcts show diffusion restriction on DWI, with hyperintensity on DWI and corresponding hypointensity on ADC[34] .
DTI is even more sensitive than DWI, especially for pontine and medullary lesions.

A negative DWI does not exclude the diagnosis of stroke. Between 2-7% of patients with a final diagnosis of stroke are initially DWI negative[34].

Very small (lacunar) infarcts, brainstem lesions, clot lysis with recanalization, and moderately reduced or fluctuating hypoperfusion that is not severe enough to restrict water movement have all been cited as possible reasons for DWI-negative acute strokes[34].

CAROTID DOPPLER STUDY-Carotid Doppler study is principal non-invasive test for assessing carotid artery narrowing. Plaques causing symptoms likely hypoechoic and produces very narrowing of vessel, plaques without symptoms are hyperechoic and produce medium stenosis.

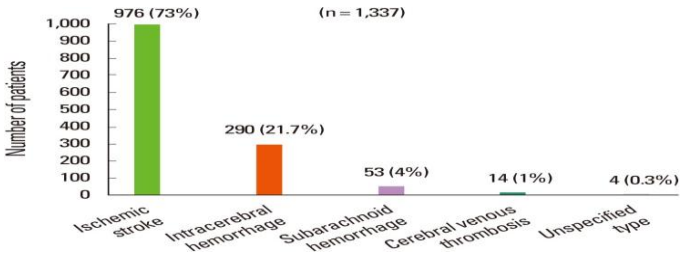


The grade of narrowing assessed depending on waveform and spectral evaluation.Blood flow is laminar in non stenotic condition.as narrowing increases,blood stream change to turbulent. Valuable sign in ultrasound is the more the grade of narrowing,velocity is higher.

Carotid stenosis 60% or more is clinically significant[36].With ultrasound, the intimal-medial thickness(IMT) of the carotid artery measured.As IMT of the carotid artery increases,risk of myocardial infarction and stroke increases

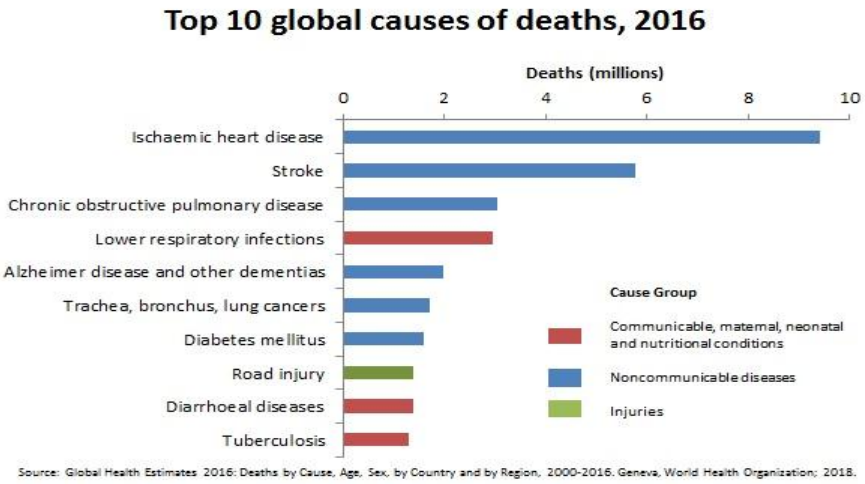
Intracranial stenosis and occlusion corresponds to approximately 8-10% of acute ischemic stroke.

EPIDEMIOLOGY



According to the WHO, world health report, 15million suffer stroke each year.Among these 5 million permanently disabled and 5 million die[37].To more than 12.7 million strokes worldwide high blood pressure is contributor About 650,000 stroke deaths each year in Europe[37].Efforts to lower blood pressure and reduce smoking, the incidence of stroke is declining in developed countries but overall rate of stroke remains towering ascribed to aging of the population[37].

Stroke has already reached epidemic proportions.By 2015,current trends indicate number of death yearly increases to 6.7 billion if no suitable action taken.



Stroke is responsible for more deaths annually than AIDS, tuberculosis and malaria combined. It now disproportionately affects individuals living in resource-poor countries. " In India, 2004,cerebrovascular accident shared 41% death and 72% disability adjusted life year determined by ICMR[38]".

"The Indian National Commission on Macro-economics and Health measured that the number of infarct cases may grow from 1,081,480 in 2000 to 1,667,372 in 2015, (Shah + Mathur 2006) The Global Burden of Disease Study projects shows in India will surpass established market economies by year 2020 in stroke deaths number[39]".

"The crude prevalence rate appears to be higher in urban compared to rural populations[39]"."(Bharucha et al 1988, Bharucha + Kuruvilla 1998) For India, the overall age adjusted prevalence rate for stroke is estimated to lie between 84- 262/100,000 in rural and between 334-424/100,000 in urban areas[40].

Overall in India, the adjusted annual incidence (per 100,000 persons) of stroke is 124 in rural area (Bhattacharya 2005)[41] and 145 in urban area (Das 2007)[42].

Individual Indian studies have estimated that the prevalence rates increases from 0.1-0.3/1000 in the < 45 year age group to 12-20/1000 in the 75-84 year age group (Prasad

2010)[43]".“Similarly, the incidence rates increase from 27-34/100,000 in the 35-44 age groups to 822-1116/100,000 in the 75+ age group (Dalal et al 2008)[44].

In India, the prevalence of stroke in younger individuals is high (18-32% of all stroke cases) compared with high-income countries[44] (Dalal et al 2008).

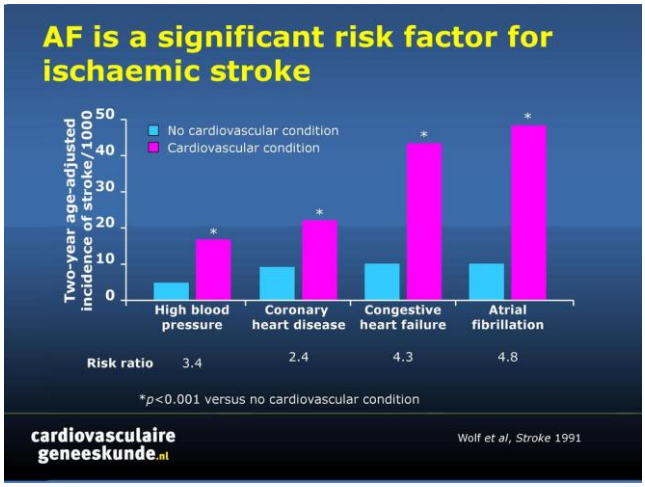
Stroke prevalence among the elderly in rural India 1.1% and urban India 1.9%. Prevalence is directly proportional to age and inversely proportional to the education levels of stroke survivors[45] (Feri 2011). However, it is believed that the average age of patients with stroke in developing countries is 15 years younger than that in developed countries[46] (Tripathi 2011).

Indian studies have shown that about 10% to 15% of strokes occur in people below the age of 40 years[47] (Feigin 2007). In India, nearly one-fifth of patients with first ever strokes admitted to hospitals are aged <40 years[48] (Pandian 2005)".

"Higher proportions of younger individuals are affected in India compared to developed countries. Ischaemic stroke is the most common subtype followed by haemorrhagic and embolic stroke and 21-48% of stroke in young is caused by atherosclerotic large artery occlusive disease [49].

Interim analysis of 3092 patients in a study (INSPIRE) conducted in India found that approximately 27% (814) of the patients with stroke below the age of 50 and 30% (935) of patients had a poorer socio economic status.Thus the higher incidence of stroke among younger age group and also among poorer population in India[49] (Xavier 2012).

This concurs with previous evidence: Das et al demonstrated that the incidence and prevalence of stroke greater among slum dwellers in Kolkata than in non-slum dwellers, but the difference was not statistically significant (Das et al 2007)[42]".



"Tripathy et al able to identify that the prevalence of risk factors such as hypertension and hypercholesterolemia 7% and 10% higher respectively among the low-income urban population of Chennai compared with the middle-income population[46] (Tripathy et al 2007).Men are more likely to have a stroke than women: the male/female sex ratio for India is 7:1 [50](Sethi 2002).This may be due to differences in risk factors such as smoking and drinking which are more prevalent among men in India compared with women[51] (Das + Banerjee 2008).

The mean onset of stroke for men in India ranges from 63-65 for men and 57-68 for women[41,44,52] (Bhattacharya et al 2005, Dalal et al 2008, and Sridharan et al 2009).

The estimated life years lost due to stroke 5,289,357 in 2004, an increase from the 1998 estimate of 4,818,740 [53](WHO 2005).

A recent study indicated that national per-capita income the strongest predictor of stroke mortality and Disability Adjusted Life Years (DALY) loss even after adjustment for cardiovascular risk factors[54] (Jeffrey 2009)".

"In 1997, estimated that 28.5 million DALYs lost due to stroke worldwide; nearly 6 times higher than that of malaria[55] (Dalal et al 2007). This is projected to increase to 61 million DALYs in 2020 and 84% of these DALYs lost will be in developing countries[55] (Dalal et al 2007).

In South East Asia alone, where India comprises 81% of the population, 6.36 million DALYs are estimated to be lost due to stroke[56] (Gupta 2008).

The economic burden caused by stroke has not been explored in India [57](Pandian et al 2007). However, India is estimated to have lost 8.7 billion dollars in 2005 due to coronary heart disease (CHD), stroke, and diabetes[53](WHO 2005)".

"This is to increase to 54 billion dollars by 2015 and India's growth of gross domestic product (GDP) is estimated to fall by 1% because of the combined economic impact of CHD, stroke, and diabetes[53] (WHO 2005).

Recent evidence suggests that 72.7% of stroke survivors in rural India have severe disability and unmet needs for stroke care [45](Feri 2011)".

BRIEF ACCOUNT OF NEURON SPECIFIC ENOLASE

During understanding the conversion of 3- phosphoglycerate to pyruvate in muscle extracts Lohman and Mayerhof discovered ENOLASE in 1934[58]

Enolase has essential need for specific divalent metal ions for function.Enolase has maximum function in existence of coenzyme magnesium[59]. Conversion of 2-phosphoglycerate to phosphoenolpyruvate catalysed by glycolytic enzyme enolase[60].

Alpha,beta,gamma are components of enzyme for its function.NSE in serum and csf have halflife of 30hrs.ENO2 gene encodes gamma-enolase or enolase 2 (ENO2) or neuron specific enolase,found in mature neurons and of neuronal origin cells,78KD gamma homodimer.

Ubiquitous enolase alpha, enolase βeta, muscle-specific and neuron specific is enolase gamma[59,60,61].

Transformation from alpha/alpha homodimer to alpha/beta heterodimer in striated muscle cells and alpha/gamma in nerve cells occur during ontogenesis[59,60,61].

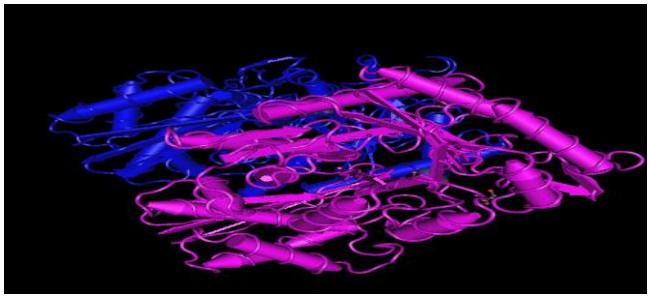
The C-terminal part of the molecule, which is not related to glycolytic pathway shown to promote survival of neuronal cells by regulating neuronal growth factor receptor dependent signalling pathways, resulting also in extensive actin cytoskeleton remodelling[62].

NSE, neurotrophic and neuroprotective properties on a broad spectrum of central nervous system (CNS) neurons.Promotes cell survival by binding in calcium dependent manner to neocortical cells cultured[63].

NSE,diffuses easily into extracellular fluid and CSF if membranes damaged and has good stability in biological fluids[64].

Neural damage in several neurological disorders including stroke, hypoxic brain damage, status epilepticus, Creutzfeldt- Jakob disease, and herpetic encephalitis,NSE has good sensitivity and useful marker clinically[64].

NEURON SPECIFIC ENOLASE STRUCTURE-



Enolase, part of grand enolase superfamily with molar mass about 82,000-100,000 Daltons changes with isoenzyme[65,66].

Two parts are antiparallel in direction leading to Glu in one part join ionic bond with Arg in other part. individual unit has two separate domains in human alpha enolase[65].

"Three α-helices and four β-sheets in smaller N-terminal domain .Two β-sheets followed by two α-helices and ends with a barrel composed of alternating β-sheets and α-helices arranged so that the β-beta sheets are surrounded by the α-helices in larger C-domain[65,67],,

Significant hydrophobic interactions between these two domains responsible for compact and globular structure[65,67].

Enolase is a extremely preserved protein with 5 working-areas residues being particularly crucial to function.In contrast to unchanged enzyme, a changed enzyme varies sometimes Glu,Glu,Lys / Lys residue will have functional status restricted by a value of 105[65].

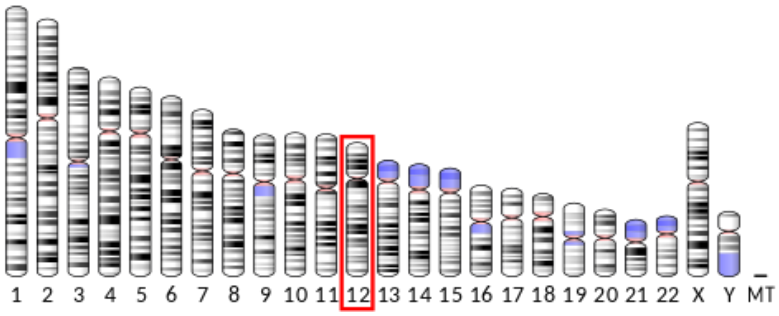
Also, modifications impacting His make changed gene enolase have just 0.01% of enzymatic function. Two coenzymes magnesium neutralizes negative charges in substances.

Human γ- or neuron-specific enolase nucleotide series from upper side to the 5′ end to further than the polyadenylation site.12 exons dispersed throughout 9213 nucleotides.

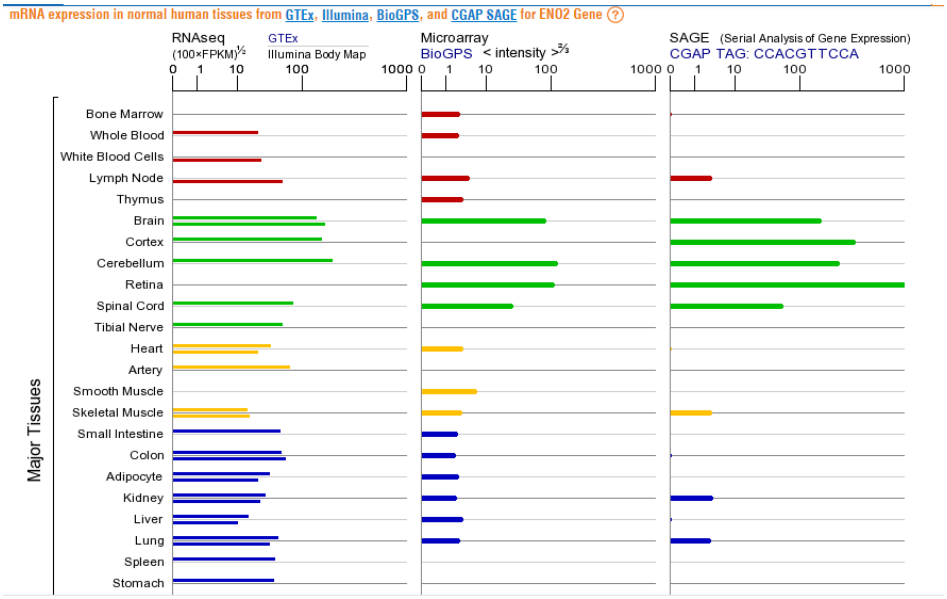
Introns happen in locations similar as documented homologous rat gene,and human α- or nonneuronal enolase gene,encouraging the presence about one predecessor among these gene group.Moreover, upside down *Alu* series exisitng around 572 nucleotides upside of

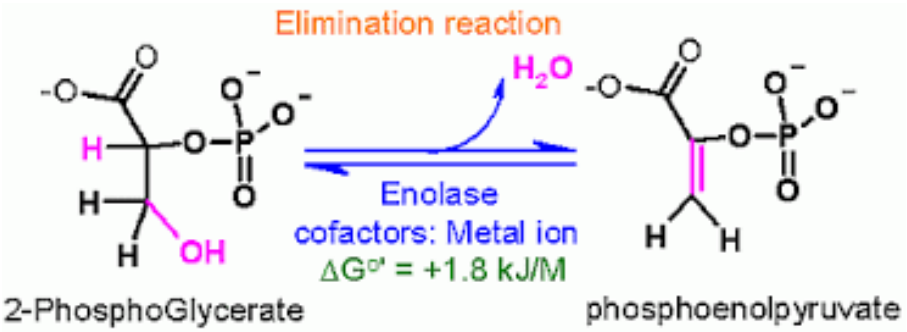
the main initiating area.Contrast 5′-flanking region in human γ-enolase gene similar area at rat gene disclosed great grade about series preservation.

In the introns 1, 5 and 10 there tandem repeats $(GT)_{33}$, $(GT)_{21}$ and $(GT)_{24}$, respectively. The 3 ' end contains a single polyadenylation site and an identifier sequence 2 kb downstream from the poly(A)-addition site.



12p13.31.>1299bp





Haemolysis causes rise in NSE in serum in ratio to the grade of rbc lysis.Serum NSE is the only serum indicator of neural damage included in protocols for brain injury prediction following heart stops beating.

"The proposed cut-off value (33 µg/L) based primarily on studies of cardiac arrest patients not treated with therapeutic hypothermia[68,69]".Serum NSE,existing higher quantities in organs like neural and neuroendocrinal organs compared to different mankind organs estimated using elisa techniques.

Serum NSE accurate site,not clear.Immunohistochemical tracking of enzyme alternative to neural & neuroendocrinal organs by ELISA techniques.

"The gamma-enolase located in media of aorta, fibromuscular tissue of the prostate, and the myometrium of the uterus, myoepithelial cells, the conducting system of heart, epithelial cells of loops of Henle, and macula densa cells of the kidney,spermatogonia,

lymphocytes, plasma cells, platelets, and megakaryocytes and in lesser amounts in bronchial epithelial cells and type II alveolar epithelial cells of the lung, and in secretory cells of the fallopian tube[70]".

Now,many commercially accessible NSE immunoassays from varies producers and no common standard.Many diagnostic and pre-diagnostic parameters impact the assessed outcome.

Example, depending on the interference of NSE from rbc specified that an index of haemolysis to be conducted before to evaluating the sample and not usually done.

Ramont et al. discovered no impact on NSE-values by the preserving of serum for 9 months , impact of longer duration preserving examined[71].

FUNCTIONS OF NEURON SPECIFIC ENOLASE-

Biological Process

small molecule metabolic process

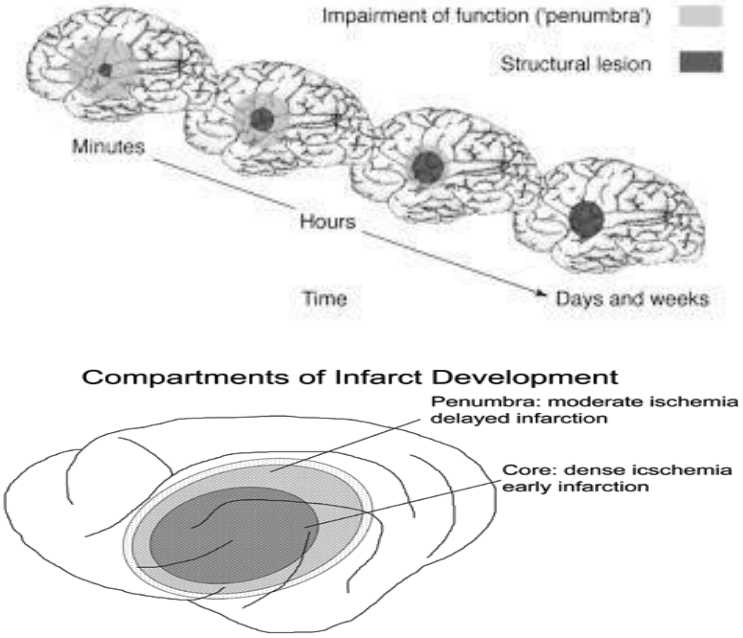
Glycolysis

response to drug

Gluconeogenesis,response to estradiol stimulus

BRIEF ACCOUNT OF INFARCT VOLUME

Infarct Development[72]



Infarct volume is one of the common indices for evaluating the extent of ischemic brain injury following focal cerebral ischemia.

Accuracy in the measurement of infarct volume is compounded by postischemic brain edema that may inflate brain volume in the infarcted region.

PENUMBRA delinated area as ischemic tissue possible designate for infarction but it isn't irrepable damaged and the focus of any treatment. The penumbra initially explained on electrophysiological basis as the tissue present between the thresholds of electrical failure and ion pump failure[73] (Astrup *et al*., 1981).

Functionally, penumbral tissue need to fulfill standards (i) operationally altered living underperfused tissue with unknown destiny that is at-danger of infarction if unsaved; (ii) participating to the functional deficiency and that (iii) resolving is related with ratio of rehabilitation of clinical weakness.

Penumbra area may stay alive for couple of hours following ischaemic incident because of collateral blood supply to penumbral area.The penumbra area particularly happens

if blood supply goes lower than 20 mL/100 g/min. At this moment electrical transmission among nerve cells breaksdown.

Cells in this area are viable but metabolic pumps are blocked, oxidative metabolism is lowered but nerve cells start to depolarize again.Region of the cerebrum usually not infarcted until blood supply to the area lower than 10 to 12 mL/100 g/min.

This moment, glutamate discharge uncontrolled,ion pumps are blocked and adenosine triphosphate (ATP) synthesis ceases leading finally to interruption of intracellular mechanisms and nerve cell death.

A greater volume of penumbra surrounding a ischaemic infarction indicates higher volume of possible saveable neural matter by clot lysis and clot removal.Such treatment leads to higher impact on recovering clinical outcome like mobility following ischaemic infarction.

NEUROLOGICAL SCALES

GLASSGLOW COMA SCALE

The glassglow coma scale explained in 1974 by Graham Teasdale and Bryan Jennett as a mode to express about the status of consciousness of sufferers with an abrupt neural damage.

The outcome utilizing the scale aid in early judgement and oversee course in pliantness that are significant in indicating the necessity for novel efforts.

Eyes	**Verbal**	**Motor**
• Orientated	Spontaneous	Obey commands
• Confused	to sound	Localising
• Words	to pressure	Normal flexion
• Sounds	none	Abnormal flexion
• None		Extension
• none		

There are three main sources of possible interference with assessment of one or more component of the scale.

1. **Pre-existing factors**
    - Language or cultural differences
    - Intellectual or neurological deficit
    - Hearing loss or speech impediment
2. **Effects of current treatment**
    - Physical e.g. intubation or tracheostomy
    - Pharmacological e.g. sedation or paralysis
3. **Effects of other injuries or lesions**
    - Orbital/Cranial fracture
    - Dysphasia or Hemiplegia
    - Spinal cord damage.

NATIONAL INSTITUTE OF HEALTH STROKE SCALE

When using the NIHSS it is valuable that the clinican donot instruct with the activity. The clinican may show the instructions to sufferers who are not able to understand oral commands,but scale value must mirror the sufferer's self capability.It is allowed for the clinican to bodily assist the sufferer to be in situation to start the procedure, but the clinican need not give additional help when the sufferer is trying to finish the activity. For every element the clinican should value the sufferer's initial task, and frequent efforts should not impact the sufferer's value.

1. Status of Consciousness

Status of consciousness examining is split into three parts. The foremost LOC component exam for the sufferer's retorting. The second LOC part is depend on the sufferer's capability to reply querries which are orally asked by the sufferrer’s. The final LOC subpart is depend on the sufferrer's capability to reply to oral instructions to do easy activity

A)LOC responsiveness-The clinican should assess if the sufferrer is completely attentive to own things around.When sufferrer is not fully attentive, the clinican should try a oral command to wake up the sufferrer. No response to oral command suggest try to wake up the sufferrer through frequent bodily inducement.When nothing of these inducement are succeed in provoking a pliantness, the sufferrer may be demmed completely callous.

Score-0=attentive,plaintness.1=Not attentive; orally wakable or awaken by small inducement to comply, reply, or retort.2-=Not attentive; just retort to frequent or powerful and hurting inducement. 3=completely not retorting; retort just with reflexes or is areflexic.

B) LOC Question-sufferrer is orally questioned own time of life and about running month.

0= appropriate respond couple of queries 1=appropriate respond to single querry 2=No appropriate response to any of querries

C) LOC Commands-The sufferrer is given oral command to foremost to open and shut own's eyes and later grasp and let go own hand .Score 0=accurately done couple of work. 1=accurately done 1 work .2= Not accurately done any of work.

2. Horizontal Eye Movement

Examine capability of sufferrer to follow a pen or finger from place to place just with help of own's eyes. This is structered to examine motor capability to look in the direction of part of cerebrum contrary of trauma. This component is examined since Conjoined visual discripancy exist about twenty percent involving cerebral infarct patients. CED is usually greater involving non-left of cerebral infarct & usually of injuries impacting the basal ganglia & temporoparietal cortex.Injuries of above said regions lead to reduced spatial alertness and decreased management of eye motions.

Score-0= usually; capable of tracing pen/digit by changing position.1= incomplete visual weakness;look not normal involving either visual fields,however look isn't completely disabled.sufferrer may look in the direction of hemisphere of stroke, but cannt cross midline.2= Total gaze paresis; gaze is fixed to one side central line.

3. Visual field test

Examine the sufferrer's eye sight in every vision area.single eye examined separately, closing single eye & later second.Every upside & downside area examined questioning sufferrer to show number of digits clinican is showing involving every area.Clinican shall command sufferrer sustain visual connection during entire exam,donot enable sufferrer reorganize concentration in direction of every inducement.Using foremost eye closed, keep a disordered amount of fingers in every quadrant and question the sufferrer the number of fingers shown. Recur this examing for the second eye

Score:0 =eye sight preserved.1=Partial hemianopia or complete quadrantanopia; sufferrer identify no visible inducement in single particular visual field.2=Complete hemianopia; sufferrer identifies no visible inducement in single part of the visible area.3=Bilateral Blindness, consisting vision loss from whatever etiology

4. Facial Weakness

Facial weakness,either incomplete/ total weakness involving face.Usually this weakness is paramount involving lower part. Injury site involved weaknes can exist in other facial areas.During scrutiny the symmetry of every facial manifestations the clinican should foremost command sufferrer to exhibit own's teeth (or gums). Later, the sufferrer should be requested to press own's eyes shut as tight as feasible.When initiating eye opening own's eyes, the sufferrer is given command to lift own eyebrows

Score:0 =Usual and symmetrical motion.1 =Little weakness; act is less than compeletely usual,like smoothened nasolabial fold or Little dysmmetry of smile .2 =Incomplete weakness; specifically weakness involving downpart of face .3 =Total facial weakness,complete weakness involving both parts of face.

5. Motor Arm

When palm looking downside,sufferrer stretch first arm 90 degrees out anteriorly,when sufferrer is sitting, and 45 degrees out anteriorly when sufferrer is supine.When required,aid the sufferrer be in the appropriate situation.After strecting sufferrer's arm is in required spot the clinican should initiate oral enumerating down from 10 meanwhile

concomittantly enumerate down on own fingers in total sight of the sufferrer.Watch to identify any downside arm ramble before the end of the 10 seconds. Downside motion happening outright after the clinican keeps the sufferrer's arm in situation should not be deemed downside ramble. Recur this exam for the other arm.

Score-0=Upper limb doesnot ramble; upper limb stays original position of total 10 seconds 1=ramble;upper limb ramble back intermediary position before ending total 10 seconds,no need of support. 2=Restricted endeavor opposing gravity; the arm is capable to regain the initial situation, but rambles downside from the starting position needing bodily aid before to the end of the 10 seconds 3=None opposition to gravity .4=None motion; sufferer dnt have capability to execute conscious motion in arm

6. Motor Leg

The sufferrer being in the lying down, aleg is kept 30 degrees over horizon. As the sufferrer's leg is in place the clinican should start orally enumerating from 5 and concomittantly enumerating down on own fingers in total display of the sufferrer.Watch whatever downward leg ramble before to the end of the 5 seconds.

Scores-0 =Lower limb doesnot ramble;lower limb stays about starting place of total 5 seconds 1=ramble;lower limb ramble about intermediary place about end of total 5 seconds, however none time in contact with bed for aid .2=restricted movement opposing gravity; the leg is capable to regain the initial situation, however rambles downside from the starting position to a bodily aid before to the end of the 5 seconds 3 =No opposition to gravity;lower limb drops suddenly following aid starting position, but sufferrer capable to move in some state (e.g. hip flex) .4 =None motion; sufferrer doesnot have capability to perform conscious motion in leg

7. Limb Ataxia

Done for the existence of a unilateral cerebellar injury, and differentiates among general weakness and lack of coordination.Sufferrer shall commanded initially reach own digit towards clinican's digit later bring that digit again towards self nasal tip,recur this

activity at frequency of three or four.Later sufferrrer shall commanded bring own heel up and down shin using own other lower limb.Above said exam shall recured using second lower limb.

Scores-0=Usual coordination; gentle and precise motion .1=Ataxia exist in single limb; inflexible and imprecise motion in single extremity .2 =Ataxia exists in 2 or more limbs: inflexible & imprecise motion involving both extremities on unilateral side.

8.Sensory

Sensory examination is done by pinprickle of proximal part involving all limbs. When pinprickimg, clinican shall question whether if or not sufferrer perceives prickle, & when perceives the pricks separately on one side in contrast to the different site.

Scores-0 =No proof of loss of sensation.1 =Medium loss of sensation; sufferrer perceives prickle, but perceives as dimmer on one site .2 =Complete loss of sensation involving single site; sufferrer unaware of felt in all single limbs

9.Language

Estimates the sufferer's language abilities. Once finishing components one to eight, usual that clinican obtained an relevance about sufferrer's vernacular abilities but significant affirming estimation same time.Infarct score consists image involving situation, a catalogue about easy sentences,image involving varies disordered things & sentences.Sufferrer shall requested describe situation at initial representation.Later read the enumerated sentences and name all of the things shown in the later representation. The valuing for each things should done depending on both the outcome from the exam done in thing in extra of vernacular abilities.

Scores-0=usual; no clear oration problem.1=Medium inability to produce speech; identifiable impairement of articulateness, but clinican able to obtain details from sufferrer's speech.2=Complete inability to produce speech; total articulation brokendown,

& clinican not able obtain picture's composition from the sufferer’s oration.3=Not able to orate or comprend oration

10. Speech

Speech defect is the loss of articulation abilities forming comprendable oration. Speech defect is rigidly a articulation difficulty doesnot associated with sufferrer's capability understand oration. Infarct that leads to dysarthria usually impact regions like anterior opercular, medial prefrontal and premotor, and anterior cingulate area,areas are important in organizing articulatory manage involving tongue, throat, lips, and lungs.For exhibiting this part sufferrer requested decipher sentences avaiable with the infarct scale meanwhile clinican watches sufferrer's articulatory and oratory fluency.0-usual oration 1-Mild-Moderate dysarthria .2-Severe dysarthria

11.Extinction and Inattention

Enough data pertaining this component collected by the clinican in components 1-10 to suitably value the sufferrer.but when confusion occurs the clinican should exam this component through a procedure called "double simultaneous stimulation". This is done by making the sufferrer near own eyes and requesting to detect the site where caress by the clinican.Meanwhile clinican changing caress sufferrer involving both sides. Later clinian caress sufferrer involving two sides for similar period. Shall recurred involving sufferrer face, arms, and legs. Examine annihilation involving eyesight clinican shall keep single digit anterior to sufferrer's eyes & request sufferrer to identify digit squirming or both squirming.Clinican shall then change among squirming each digit & squirming together for similar period. 0=usual; patient appropriately replies all querries

Scores-1=Negligence on one side involving single sensory faculty; vision, touch, hearing/spatial**.**2=Half-negligence; cannot identify inducement involving greater than single sensory faculty involving same side

MODIFIED RANKIN SCALE

The score primary provided by Dr. John Rankin of Stobhill Hospital, Glasgow, Scotland later changed for presently admitted state by Prof. C. Warlow's group at Western General Hospital in Edinburgh.Change was adding 0 to primary score meaning none symptom. The foremost issue of the present mRS 1988 by van Swieten, *et al.*

Interobserver authenticity of mRS increased with help of a organized querries throughout the interrogation mechanism, and getting evaluaters endure a varies method practice sessions.

The mRS is regularly condemed for its individual expression observed as distorting outcome, however applied every hospital process to examine recovery fulfilment and outpatient conduit. These condemed explained by investigators forming organized interrogation which demands easy querries both the sufferrer and/or the patient helpgiver may pliant to.

The Modified Rankin Scale (mRS)

The score covers zero to six,covering no complaints towards demise.

0= None complaints.1= None important unability. Able do routine activities, inspite few complaints. 2= minor unability. can do self activities with no aid, however notable to do all former works. 3= Moderate unability. need little aid however can walk unaided. 4 =Moderately severe unability. Not able to do self physical requirement with no help, and not able to walk unaided. 5= Severe unability. Need continuous nursing care and consideration, bedridden, incontinent. 6 =Dead.

AMERICAN HEART ASSOCIATION/AMERICAN STROKE ASSOCIATION 2018 GUIDELINES FOR EARLY MANAGEMENT OF ACUTE ISCHAEMIC STROKE[74]

1.Hypotension & hypovolemia readdressed to preserve systemic perfusion status essential for organ function.

2. Sufferrers having hypertension but entitled for management with IV alteplase get hypertension watchfully reduced to maintain systolic BP <185 mmHg and diastolic BP <110 mmHg prior to IV fibrinolytic therapy started.

3. sufferrers entitled for abrupt treatment but BloodPressure >185/110 mmHg

"Labetalol 10–20 mg IV over 1–2 min, may repeat 1 time; or Nicardipine 5 mg/h IV, titrate up by 2.5 mg/h every 5–15 min, maximum 15 mg/h; when desired BP reached adjust to maintain proper BP limits;orClevidipine 1–2 mg/h IV, titrate by doubling the dose every 2–5 min until desired BP reached; maximum 21 mg/h.Other agents (eg, hydralazine, enalaprilat) may also be considered"

When BP is not ≤185/110 mmHg,not to dispense alteplase Treatment of hypertension throughout and later alteplase or different abrupt treatment to keep BP ≤180/105 mmHG.Record BP every 15 min for 2 h from the initiation of alteplase treatment, then every 30 min for 6 h, and then every hour for 16 h.

When systolic BP >180–230 mmHg or diastolic BP >105–120 mmHg:

Labetalol 10 mg IV followed by continuous IV infusion 2–8 mg/min;orNicardipine 5 mg/h IV, titrate up to desired effect by 2.5 mg/h every 5–15 min, maximum 15 mg/h;orClevidipine 1–2 mg/h IV, titrate by doubling the dose every 2–5 min until desired BP reached; maximum 21 mg/h.If BP not controlled or diastolic BP >140 mmHg consider IV sodium nitroprusside

4.Origin of hyperthermia (temperature >38°C) recognized and managed,and antipyreticdrugs dispensed to reduce temperature in hyperthermic sufferrers with ischaemic infarct.

5.Proof shows that consistent in-hospital raised blood sugars within the foremost 24 hours after ischaemic infarct. Related with poor results than normal blood sugars and thus,it is sensible to manage raised blood sugars to attain blood glucose values in a extent of 140 to 180 mg/dL and to nearly record to abort hypoglycemia in ischaemic stroke.

Hypoglycemia (blood glucose <60 mg/dL) managed in sufferrers of ischaemic infarct.

7. IV alteplase (0.9 mg/kg,maximum dose 90 mg over 60 minutes with first 10% of dose dispensed as bolus over (1 minute) considered for chosen sufferrers treated within 3 AND 4.5hrs of cerebral infarct complaint initiation.

INDICATIONS-a.Time of initiation within 3hrs and 4.5hrs b.age>or <80yrs c.serious infarct complaints with haemorrhagic transformations d.previous antiplatelet treatment e.BP<185/110mmHG.f.Blood glucose >50mg/dl. End stage renal disease on hemodialysis CONTRAINDICATIONS-a.Time of onset >3-4.5hrs b.acute intracranial haemorrhage on CT.c.ischaemic stroke serious head trauma,intracranial/spinal surgery within 3months.d.Lmwh within previous 24hrs.

8. Sufferrers receive mechanical thrombectomy with a stent retriever with following criteria (1) prestroke mRS score of 0 to 1; (2) causative occlusion of the internal carotid artery or MCA segment 1 (M1) major intracranial occlusion”

9. Tenecteplase dispensed as a 0.4-mg/kg single IV bolus has not been proven to be superior or noninferior to alteplase but may deemed as an substitute to alteplase in sufferrers with minor neurological impairment and no major intracranial occlusion.

MATERIALS AND METHODS

SOURCE OF DATA:

Material for the current work will be collected from patients who attend outpatient depar tment and inpatient department in BLDEU's Shri.B.M.Patil medical college hospital and research centre,vijayapur over a period 2years from November 2017 to june 2019.
The sample size was 94 of which 47 were acute ischaemic stroke patients were studied as cases and 47 non acute ischaemic stroke patients were controls and there NSE, Glasglow coma scale, national institutes of health stroke scale, modified rankin scale and infarct volume 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.

INCLUSION CRITERIA:

CASE:

Patients with acute ischaemic stroke.Age>21years.Presenting within 72hrs of onset of symptoms.

CONTROLS:

None physical manifestations indicative about acute ischaemic stroke of age and sex matched.

EXCLUSION CRITERIA FOR SUBJECTS IN STUDY GROUP

Central nervous system infective diseases.CVA longer than 72hrs & Peripartum CVA.

Past history of head trauma,history of nervous system tumor, subarachnoid haemorrhage or other neurological disorders and or within 3months before admission.

Haemolytic specimens in which platelet or erythrocyte lysis influenced the laboratory results.

Past history of transient iscahemic attack,major cardiac,renal and hepatic diseases.

Any systemic or local disease likea.Malignancy,idiopathic intracranial hypertension.b)Diabetes mellitus type 2causing any other neurological conditions like neuropathy,retinopathy,or any other brain damage and systemic disorders that may affect parameters in study.c.Hypertension causing retinopathy,visual loss,papilledema or any other brain damage.

Status epilepticus,creutzfeld-jackob's disease,multi-infarct dementia, brain metastases .

Blood sampling and preparation of serum:

Approximately 5ml of blood obtained by "venipuncture of anticubital vein forearm of each subject in[29]" serum separator tubes under aspectic conditions within 72hrs of onset of symptoms, centrifuged at15,000rpm for 15mins then aliquotted into 2ml tubes.

Serum samples stored at - 20 degree Celsius until assay ran to evaluate .Care was taken to exclude the hemolysed serum.

"ESTIMATION OF NEURON SPECIFIC ENOLASE[75]:

Size: 96T **Range:** 1.25 ng/ml- 20 ng/ml **Sensitivity** 0.1 ng/ml

Storage and Expiration: Store at 2-8 degree celsius for 6 months.

Application: For quantitative detection of NSE in human serum, plasma, cell culture supernatant or tissue homogenate.

PRINCIPLE:

This kit was based on standard sandwich enzyme-linked immunesorbentassay technology.The purified anti- NSE antibody was pre-coated onto 96-well plates. and the HRP conjugated antinse antibody was used as detection antibodies.

The standards, test samples and HRP conjugated Detection antibody were added to wells,subsequently,mixed and incubated, then, unbound conjugates were washed away with wash buffer.

TMB substrates (A & B) were used to visualize HRP enzymatic reaction.TMB was catalyzed byHRP to produce a blue color product that changed into yellow after add ing acidic stop solution. The density of yellow is proportional to the NSE amount of sam ple captured in plate.Read the O.D.absorbance at 450nm in a microplate reader,and then the concentrationof NSE can be calculated.

Kit components

1. One 96-well plate pre-coated with antihuman NSE antibody
2. Standard: 0.6ml (40 ng/ml)
3. Sample diluent buffer: 30 ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Biotin conjugated anti- human NSE antibody (RTU): 1.5 ml
6. HRP-conjugated reagent: 6 ml
7. Stop solution: 6 ml

8. TMB substrate A: 6 ml

9. TMB substrate B: 6 ml

10. Plate sealer: 2.Hermetic bag:1

11. 37 degree celsius incubator

12. Microplate reader (wavelength: 450nm)

13. Precise pipette and disposable pipette tips

14. Automated plate washer

15. ELISA shaker

16. 1.5ml of Eppendorf tubes

17. Absorbent filter papers

18. Plastic or glass container with volume of above 1L

PROCEDURE:

1. Wash buffer

Dilute concentrated Wash buffer (Kit Component 4) 30-fold (1:30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

2. Standard

Dilution of Standard: Label 5 Eppendorf tubes with 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml respectively, add 100µl of Standard diluent buffer into eachtube, then, add 100µl of Standard (KitComponent 2) to the 1st tube and mix thoroughly; transfer 1 00µl from the 1st tube to the 2nd tube and mix thoroughly; then transfer 100µl from the 2nd tube to the 3rd tube, then transfer 100µl from the 3rdtube to 4th tube, then transfer 1 00µl from the 4th tube to the 5th tube, then aspirate 100µl from 5th tube and discard.

3. Equilibrate kit components for 15-30 min to room temperature (21-26 degree celsius).

4. Set standard, test sample and control (zero) wells on the precoated plate respectively, and then,record their positions. Add 50µl of diluted standards (20 ng/ml, 10 ng/ml, 5 ng/ ml, 2.5 ng/ml, 1.25ng/ml) into the standard wells. It is recommend to measure each stan dard in duplicate. Add 50µl of Standard diluent buffer (Kit Component 3) into the contro

l (zero) well. Do not add sample, biotin conjugated antibody and HRP conjugated reagen t into the control (zero) well.

5. For test sample wells, add 40µl of sample first, then, add 10µl of Biotin conjugated an tihuman NSE antibody (Kit Component 5). Add the solution at the bottom of each well without touching the side wall.Shake the plate mildly to mix thoroughly.

6. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37 degree celsiu s for 30 min.

7. Remove the sealer, and wash plate using one of the following methods:

Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1×) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and cla p the plate on absorbent filter papers. Repeat this procedure four more times for a totalof FIVE washes.

Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer (1×).

After the final wash, invert plate, and clap the plate on absorbent filter papers until no m oisture remained. It is recommended that the washer be set for a soaking time of 10 seco nds or shaking.

8. Add 50µl of HRP conjugated reagent (Kit Component 6) into each well (except contro l well).

9. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37 degree celsiu s for 30 min.

10. Remove the sealer, and wash the plate.

11.Add 50µl of TMB chromogenic reagent A (Kit Component 8) into each well, and the n, add 50µl of TMB chromogenic reagent B (Kit Component 9), vortex gently the plate

on ELISA shaker for 30seconds (Or shake gently by hand for 30 seconds), and incubate in dark at 37°C for 15 min. The shades of blue can be seen in the wells.

12. Add 50µl of Stop solution (Kit Component 7) into each well and mix thoroughly. Th e color changes into yellow immediately.

13.Read the O.D. absorbance at 450nm in a microplate reader within 15 min after addin g the stop solution.
For calculation, (the relative O.D.450) = (the O.D.450 of each well) (the O.D.450 of Zer o well). The standard curve can be plotted as the relative O.D.450 of each standard solut ion (Y) vs. the respective,concentration of the standard solution (X).The human NSE co ncentration of the samples can be interpolated from the standard curve.

Precautions

1. Before the experiment, centrifuge each kit component for several minutes to bring do wn all reagents to the bottom of tubes.

2. It is recommend to measure each standard and sample in duplicate.

3. Do not let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.

4. Do not reuse pipette tips and tubes to avoid cross contamination.

5. Do not use the expired components and the components from different batches.

6. Store the TMB substrate A & B (Kit Component 8 & 9) in dark.

7. Prolong the incubation time if the hypochromasia obtained. Heat the water in the wate r bath during diluting if the crystalloid appeared in Wash buffer (Kit Component 4).

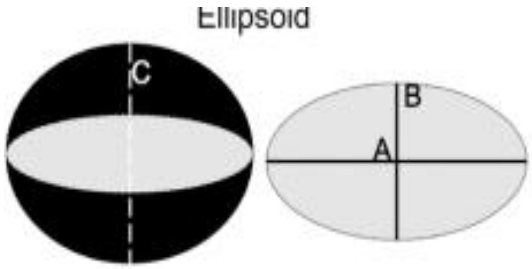
8. Do not remove microplate from the storage bag until needed, and the unused strips should be stored at 2- 8 degree celsius in their pouch or the provided Hermetic bag (Kit Component 11)"

ESTIMATION OF INFARCT VOLUME

"MRI lesions of acute or subacute volumes estimated on DiffusionWeighted Image. The ischemic Region of interest ocularly metamericed for estimating the volume. Infarct volumes extended measured from 1.4 - 525 mm^3.

The slice with the largest lesion first selected by eye. The longest lesion axis on this slice measured with the vernier callipers. Second line drawn perpendicular to first at the widest dimension.These two measurements called x (A) and y (B) axes. A third axis,the z(C) axis,computed by multiplying the number ofslices by slicethickness

CT machine used Siemens somatomscope 16/32 slice, scan slice for CT 1.5 mm.MRI machine used Philips ACHIVA 1.5tesla slice thickness 5mm.Time to perform these three measurements less than 1 minute[76]"..



$V=4/3\pi r_A r_B r_C = 4/3 \times 3 \times (A/2) \times (B/2) \times (C/2) = ABC/2$
A= longest dimension in axis x
B= longest perpendicular dimension to axis x
C= total length in z dimension

Ellipsoid model[76]:

$V = 4/3\pi\ r_A r_B r_C = 4/3 \times 3 \times (A/2) \times (B/2) \times (C/2) = ABC/2$

where A = longest dimension in axis x, B = longest perpendicular dimension to axis x (y), and

C = total length in z dimension"

3)**ESTIMATION OF WORSENING OF SEVERITY**

Stroke severity rating on day 1 & following 7th day done by clinican with help of Glasgow coma scale. Early neurological worsening identified as decrease in score by one or more points between day 1 & day 7th & stayed stable/renovated during same period categorized as no worsening.

i.e worsening of severity=score of GCS at admission-score of GCS at 7^{th} day

4)**ESTIMATION OF NEUROLOGICAL WORSENING USING NIHSS**.

The degree of disability rated at day1& following 7th day by clinican with the help of NATIONAL INSTITUTES OF HEALTH STROKE SCALE.Early neurological worsening identified as an increase in (NIHSS) by two or more points (or stroke related death) between day1 & day 7. Stayed stable /improved during same period categorized as no worsening.

i.e neurological worsening=score of NIHSS at admission-score of NIHSS at 7^{th} day.

5) **ESTIMATION OF FUNCTIONAL NEUROLOGICAL OUTCOME**.

Functional neurological outcome rated at day 1 & following 7^{th} day by clinican with help of modified rankin scale (mRS). Early functional worsening identified as negative value in mRS by one/more points between day1 & 7^{th} day. Who stayed stable /improved during same period categorized as no worsening.

i.e neurological worsening=score of mRS at admission-score of mRS at 7^{th} day

STATISTICAL ANALYSIS

The data obtained were entered in a Microsoft Excel sheet, and statistical analysis was p erformed using statistical package for the social sciences (Verson 17).

Results are presented as Mean±SD, counts and percentages. Results were compar ed using Independent t test and Mann Whitney U test.

The spearman's correlation coefficient was calculated to measure the relationship between the variables.

For all tests, significant was achieved at $p<0.05$

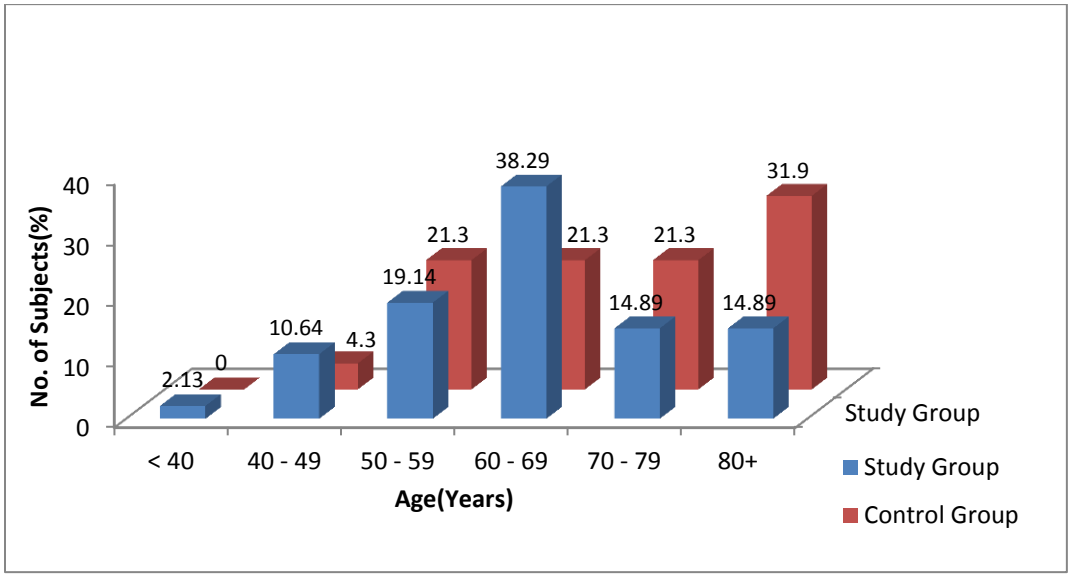
Receiver operating characteristic curve (ROC) was computed to find optimal cut off of serum NSE value, to calculate diagnostic ability of test. Chi square test used to find relation between the variables

RESULTS

In total of 94 patients ,47 cases of acute ischaemic infarct included in study group and 47 subjects as controls.

Table 1: Distribution of subjects according to Age(Years)

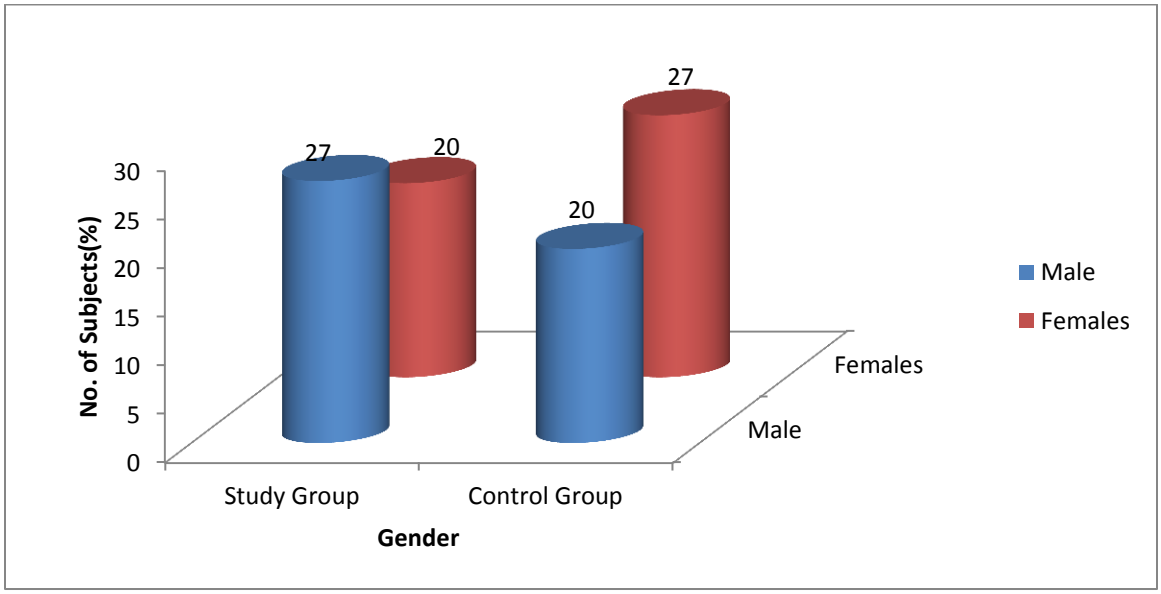
Age(Years)	Study group		Control group	
	No.of Subjects	Percentage	No.of Subjects	Percentage
<40	1	2.13	0	0
40–49	5	10.64	2	4.3
50–59	9	19.14	10	21.3
60–69	18	38.29	10	21.3
70–79	7	14.89	10	21.3
80+	7	14.89	15	31.9
Total	47	100	5	10.6



Maximum patients in study group (38.29%) in age group of 60-69 years and minimum (1%) in age group below 40years.Maximum (31.9%) in age group above,80 years and minimum (0%) in age group below 40years in control group.

Table 2:**Distribution of subjects according to gender.**

Gender)	Study group		Control group		Chi square test	P value	Remark
	No. of Subjects	percentage	No. of Subjects	percentage			
Male	27	57.4	20	42.6	X^2=2.085	P=0.1487	NS
Females	20	42.6	27	57.4			
Total	47	100	47	100.0			
NS: Not Significant							



In study group,percentage of males (57.4%) more compared to females(42.6%)

Table 3: 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 4: 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.

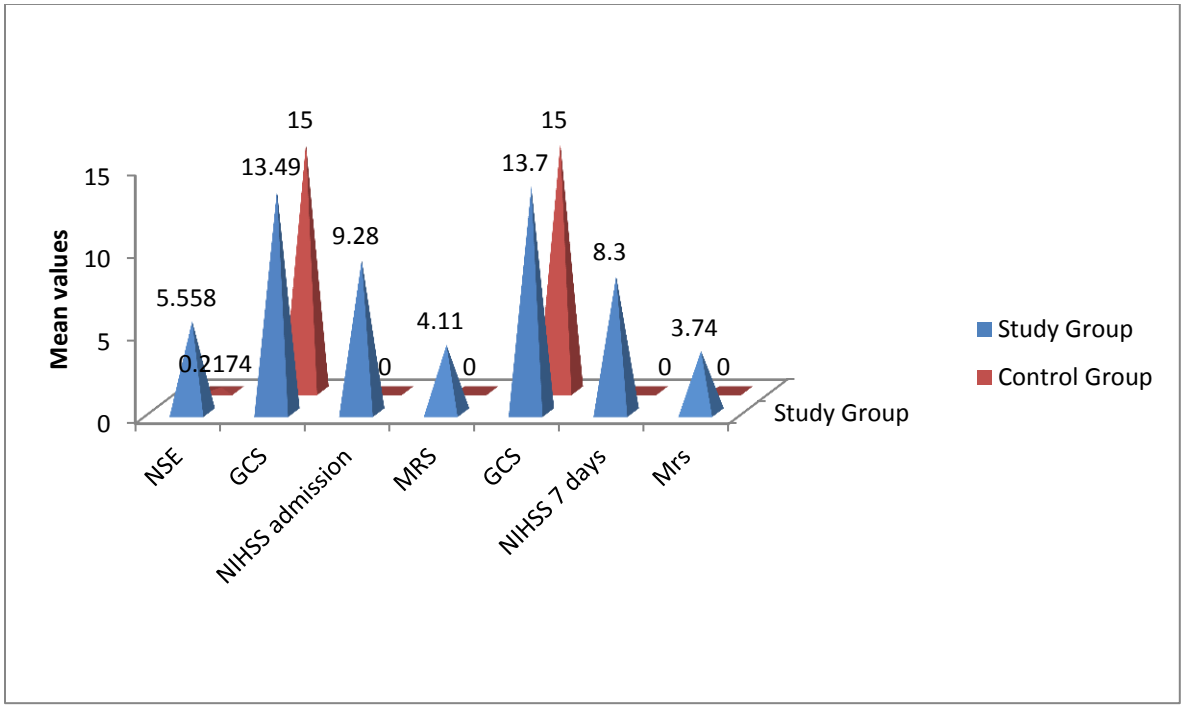
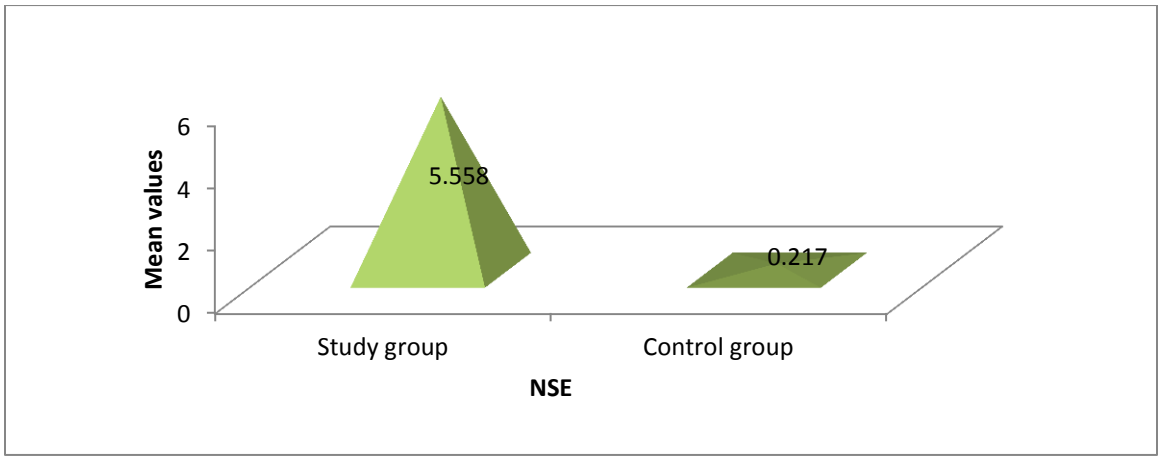
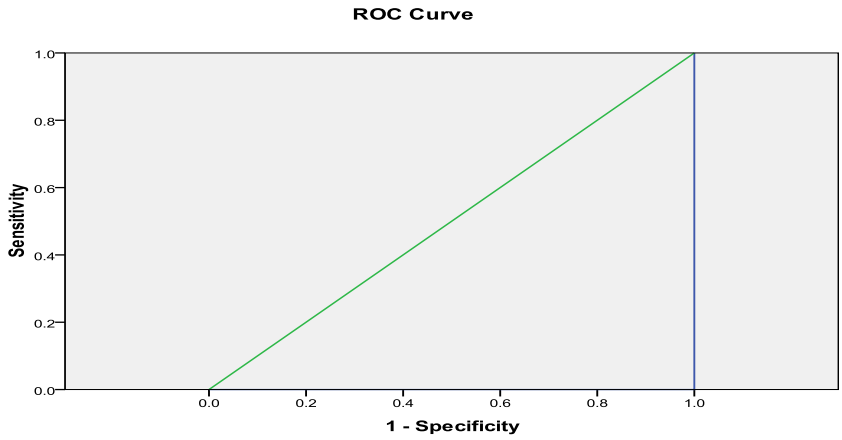


Table 6: Comparison of NSE Between Study and Control Groups

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 val ue <0.0001 statisically 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**

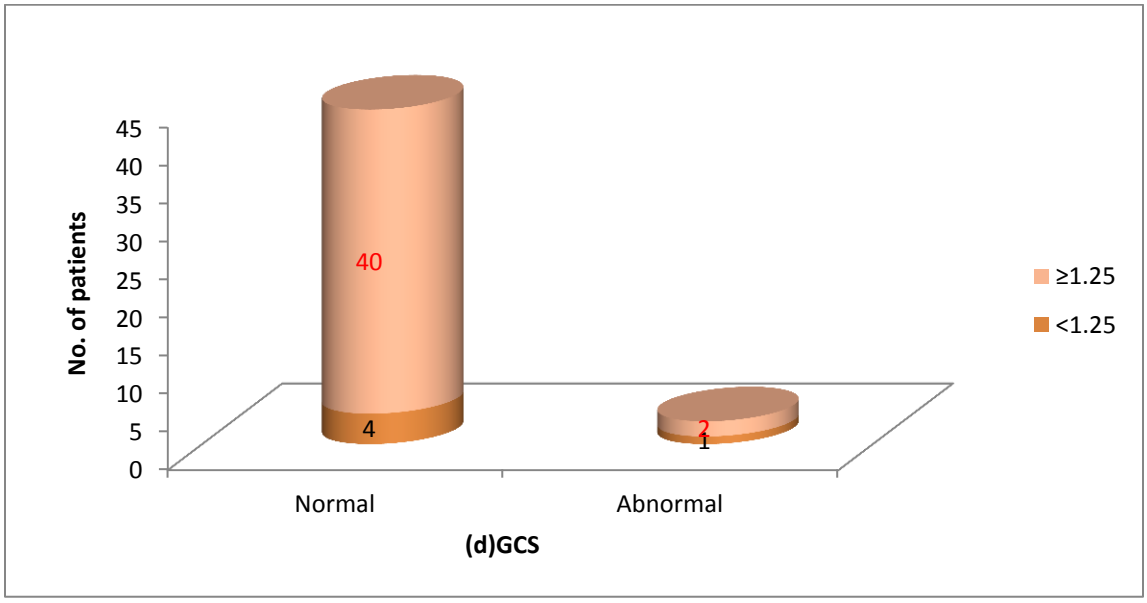
NSE	**Study group**	**Control group**	**Chi square test**	**P value**
<1.48	5	45	X^2=68.365	P<0.0001*
≥1.48	42	02		
***: Highly significant**				

Table 7:

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

Table 8: Association between NSE and (d)GCS

NSE	(d)GCS			
	Normal	Abnormal	Fisher's exact test	Remark
<1.25	4	1	P=0.2920	NS
≥1.25	40	02	NS	
NS: Not significant				

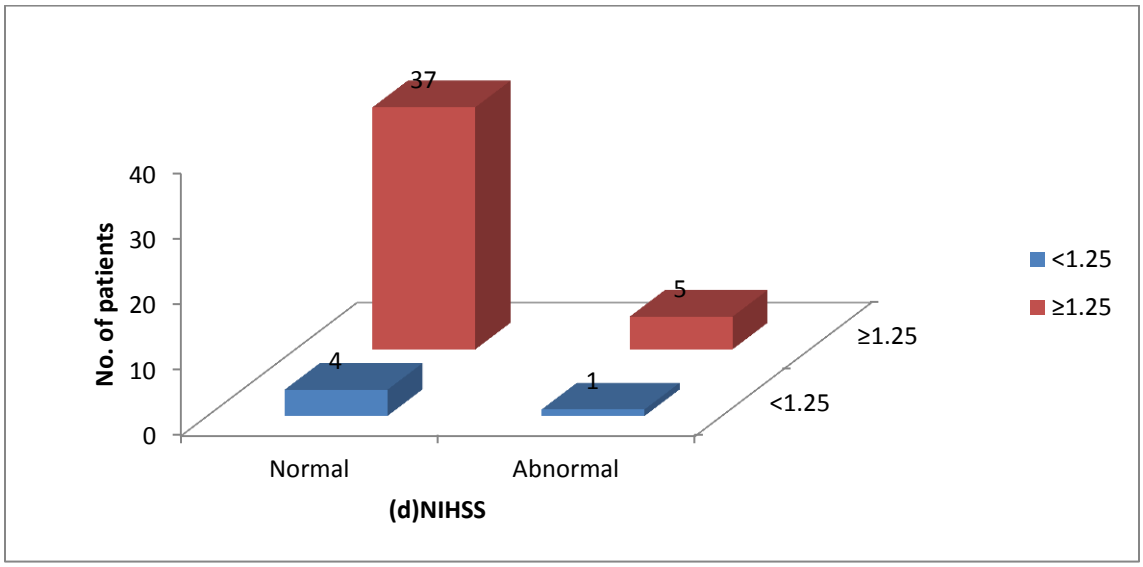


In present study,No significant association between NSE and (d)GCS.

Table 9: 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		

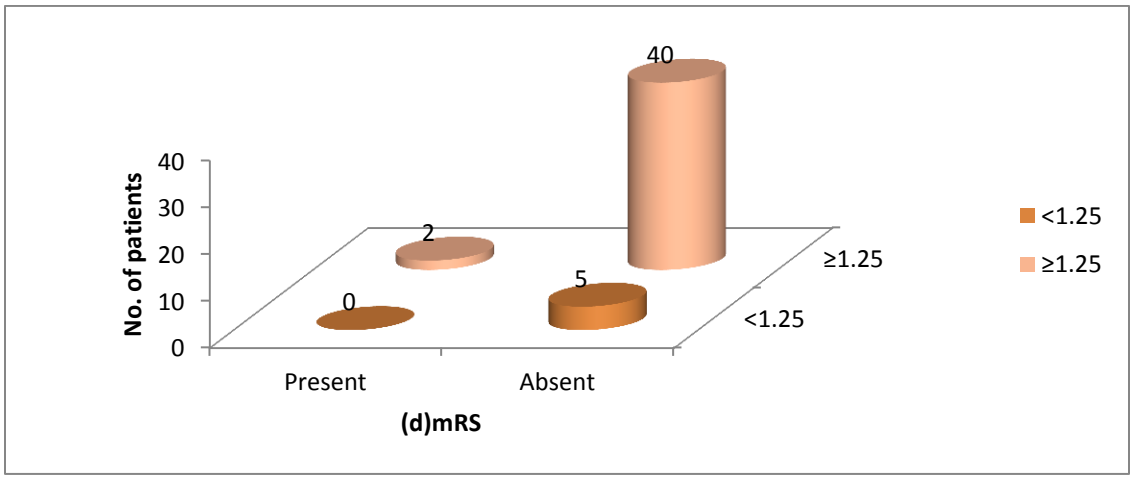
HS: Highly significant



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

Table 10: Association between NSE and (d) mRS.

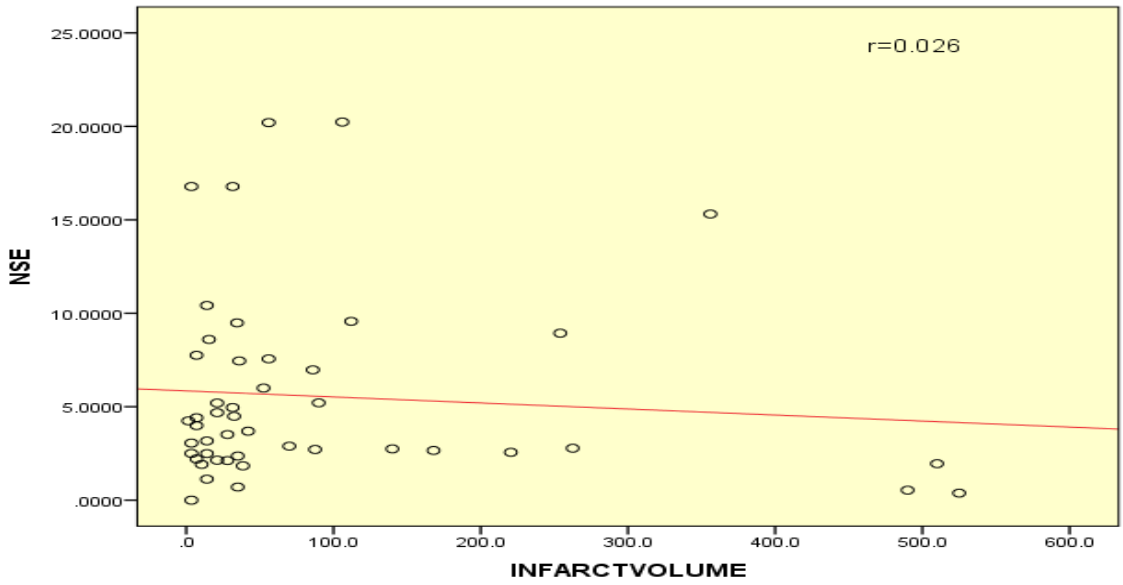
NSE	(d)mRS			
	Present	**Absent**	**Fisher's exact test**	**Remark**
<1.25	0	5	P<0.001	HS
≥1.25	2	40		
HS: Highly significant				



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

TABLE 11: Correlation Between NSE and Infract Volume

Correlation between	Spearman's Correlation 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 v olume with spearman's correlation coefficient r=0.026 but statistically insiginificant 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 assessedby 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 sufferrers and useful marker for acute ischemic stroke.

1)**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 [24](2012), Padalkar Ramchandra K et al [29] (2014).

SERUM NSE	Anuradha bharosay et al[24]	Padalkar Ramchandra k et al[29]	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

2) **DIAGNOSTIC PERFOMANCE 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.

Diagnostic performance of NSE	Padalakar ramchandra K et al[29]	Natheer H Rawi et al[21]	Hill et al[72]	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%[72].

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%)[21].

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[29]

3) **CORRELATION BETWEEN SERUM NSE AND GCS**

Correlation between NSE an d (d)GCS	Missler's et al[73]	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[23].

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 GCS[73].

4) **CORRELATION BETWEEN SERUM NSE AND NIHSS**

(degree of disability/severity of stroke)

Correlation between NSE and (d)NIHSS	González García *et al*[21]	oh et al[15]	Wu *et Al*[18]	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[21].

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)[15]. 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)[18]".

5) CORRELATION BETWEEN SERUM NSE AND mRS

Correlation between NSE and (d) mRS	Brea et al[74]	Wunderlich et al[75]	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[74].

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 done[75]".

6) **CORRELATION BETWEEN NSE AND INFARCT VOLUME**

Correlation between NSE and infarct volume	Brea et al[73]	Oh et al[15]	Sana zaheer et al[26]	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 4^{th} and 7^{th} days (Spearman coefficient 0.456)[73].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)[15] .Zaheer s et al positive correlation found between concentration of NSE on day 1 and infarct volume determined by CT scan (r =0.955)[26] 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.

SUMMARY

Acute ischaemic stroke is a major public health problem worldwide.In india,incidence is increasing rapidly.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 neurobiochemical marker like NSE release in circulation assist to evaluate pathogenesis and prognosis in patients with stroke.These NSE values are not only quantitative but qualitative values which are potentially diagnostic.

In this study,the patients admitted to BLDEU's Shri B.M.Patil medical college hospital and research centre,Vijayapur were selected and total number of 94 subjects studied which included 47 acute ischaemic stroke patients as cases and 47 nonacute ischaemic stroke patients as controls.

There was significant increase in serum NSE levels in cases as compared to controls, significant diagnostic accuracy of 100% sensitivity and can be of use for foreseeing degree of disability & initial functional neurological aftereffect and infarct volume.

This study signifies the sensitivity of NSE in acute iscahemic infarct patients contrast of nonischaemic stroke patients and early intervention of these patients will reduce the morbidity and mortality associated with iscahemic stroke.

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ETHICAL CLERANCE CERTIFICATE

ANNEXURE 7

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I the undersigned----------S/O.D/O.W/O.----------
------aged ----------years ordinarily resident of----------do here by state/declare that Dr.Sagar.S.Rashinkar of Shri B.M.Patil medical college and hospital has examined me thoroughly on----------at----------(place) and has explained to me in my own language ----------that I am suffering from----------disease(condition) and this disease/condition mimic following diseaases----------Further Dr.Sagar.S.Rashinkar informed me that he is conducting dissertation/research titled "**Study OF NEURON-SPECIFIC ENOLASE as potential Biomarker for Assessing the Severity and Outcome in patients with ACUTE ISCHAEMIC STROKE".**Under the guidance of Dr.P.G.Mantur requesting my participation in my study.Further Doctor has informed me that my participation in this study help in evaluation of results of the study which is useful reference for treatment of other similar cases in near future ,and also I may be benefited in getting relieved of suffering or cure of disease I am suffering.

The Doctor has also informed me that information given by me,observations made/photographs/videographs taken upon me by the investigator will be kept secret and not accessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary based on information given to me,I can ask any clarification during the course of treatment/study related to diagnosis,procedure of treatment,result of treatment or prognosis..At same time I have been informed that I can withdraw from my participation in this study at any time if I want or investigator can terminate me from study at any time from the study but not the procedure of treatment and followup unless I request to discharge.

After understanding the nature of dissertation or research,mode of treatment I the under signed Shri/Smt----------under my full conscious state of mind I agree to participate in the said research/dessertation.

Signature of patient:

Signature of Doctor:

Witness 1

Witness 2

ANNEXURE 8

B.L.D.E.U'S SHRI B.M.PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,VIJAYAPUR,KARNATAKA

SCHEME OF CASE TAKING

PATIENT NAME: I.P. NUMBER:

AGE: SEX:

ADDRESS: OCCUPATION:

DATE OF ADMISSION: DATE OF DISCHARGE:

SYMPTOMS::

1.Fever yes/no
2.Preceding delivery yes/no
3.Preceding convulsions yes/no
4.Preceding headache yes/no
5.Unconsciousness yes/no
6.Right sided hemiplegia yes/no
7.Left sided hemiplegia yes/no
8.Monoplegia yes/no

TREATMENT HISTORY:

Use of any drug altering serum nse levels:

Personal history:

Habbits:h/o smoking yes/no

H/o alcohol consumption yes/no

Past history:

Any medical disease affecting serum NSE levels:

Physical examination:

GENERAL CONDITION:

Pallor: yes/no

Icterus : yes/no

Lymphadenopathy: yes/no

Cyanosis : yes/no

Clubbing : yes/no

Edema : yes/no

VITALS:

Temperature:

Pulse:

Respiratory rate:

Blood pressure:

Pt condition on discharge: improved/worsened/same/expired

SYSTEMIC EXAMINATION

C.N.S:

C.V.S:

P.A:

R.S:

LABORATORY INVESTIGATIONS:

CBC

RBS

BLOOD UREA/SERUUM CREATININE

LIPID PROFILE

URINE EXAMINATION

ECG

FUNDOSCOPY

CT/MRI BRAIN

2D ECHO

SERUM NSE

DIAGNOSIS:

GLASGOW COMA SCALE

Patient Name: _____
Rater Name: _____
Date: _____

Activity		Score
EYE OPENING		
None	1 = Even to supra-orbital pressure	
To pain	2 = Pain from sternum/limb/supra-orbital pressure	
To speech	3 = Non-specific response, not necessarily to command	
Spontaneous	4 = Eyes open, not necessarily aware	_____
MOTOR RESPONSE		
None	1 = To any pain; limbs remain flaccid	
Extension	2 = Shoulder adducted and shoulder and forearm internally rotated	
Flexor response	3 = Withdrawal response or assumption of hemiplegic posture	
Withdrawal	4 = Arm withdraws to pain, shoulder abducts	
Localizes pain	5 = Arm attempts to remove supra-orbital/chest pressure	
Obeys commands	6 = Follows simple commands	_____
VERBAL RESPONSE		
None	1 = No verbalization of any type	
Incomprehensible	2 = Moans/groans, no speech	
Inappropriate	3 = Intelligible, no sustained sentences	
Confused	4 = Converses but confused, disoriented	
Oriented	5 = Converses and oriented	_____

TOTAL (3–15): _____

References

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N I H STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

Time: ___ ___:___ ___ [ ]am [ ]pm

Person Administering Scale _____

Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).

Instructions	Scale Definition	Score
1a. Level of Consciousness: The investigator must choose a response if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation.	0 = **Alert;** keenly responsive. 1 = **Not alert**; but arousable by minor stimulation to obey, answer, or respond. 2 = **Not alert**; requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped). 3 = Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, and areflexic.	_____
1b. LOC Questions: The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier, or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues.	0 = **Answers** both questions correctly. 1 = **Answers** one question correctly. 2 = **Answers** neither question correctly.	_____
1c. LOC Commands: The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to him or her (pantomime), and the result scored (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored.	0 = **Performs** both tasks correctly. 1 = **Performs** one task correctly. 2 = **Performs** neither task correctly.	_____
2. Best Gaze: Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored, but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI), score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness, or other disorder of visual acuity or fields should be tested with reflexive movements, and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy.	0 = **Normal.** 1 = **Partial gaze palsy;** gaze is abnormal in one or both eyes, but forced deviation or total gaze paresis is not present. 2 = **Forced deviation,** or total gaze paresis not overcome by the oculocephalic maneuver.	_____

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NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

3. Visual: Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat, as appropriate. Patients may be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia, is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction, patient receives a 1, and the results are used to respond to item 11.	0 = **No visual loss.** 1 = **Partial hemianopia.** 2 = **Complete hemianopia.** 3 = **Bilateral hemianopia** (blind including cortical blindness).	_____
4. Facial Palsy: Ask – or use pantomime to encourage – the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barriers obscure the face, these should be removed to the extent possible.	0 = **Normal** symmetrical movements. 1 = **Minor paralysis** (flattened nasolabial fold, asymmetry on smiling). 2 = **Partial paralysis** (total or near-total paralysis of lower face). 3 = **Complete paralysis** of one or both sides (absence of facial movement in the upper and lower face).	_____
5. Motor Arm: The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine). Drift is scored if the arm falls before 10 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in the case of amputation or joint fusion at the shoulder, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** limb holds 90 (or 45) degrees for full 10 seconds. 1 = **Drift;** limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed or other support. 2 = **Some effort against gravity;** limb cannot get to or maintain (if cued) 90 (or 45) degrees, drifts down to bed, but has some effort against gravity. 3 = **No effort against gravity;** limb falls. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **5a. Left Arm** **5b. Right Arm**	_____ _____
6. Motor Leg: The limb is placed in the appropriate position: hold the leg at 30 degrees (always tested supine). Drift is scored if the leg falls before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime, but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic leg. Only in the case of amputation or joint fusion at the hip, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice.	0 = **No drift;** leg holds 30-degree position for full 5 seconds. 1 = **Drift;** leg falls by the end of the 5-second period but does not hit bed. 2 = **Some effort against gravity;** leg falls to bed by 5 seconds, but has some effort against gravity. 3 = **No effort against gravity;** leg falls to bed immediately. 4 = **No movement.** UN = **Amputation** or joint fusion, explain: _____ **6a. Left Leg** **6b. Right Leg**	_____

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N I H STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

		____
7. Limb Ataxia: This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, ensure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position.	0 = **Absent.** 1 = **Present in one limb.** 2 = **Present in two limbs.** UN = **Amputation** or joint fusion, explain: _____	_____
8. Sensory: Sensation or grimace to pinprick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms [not hands], legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total sensory loss," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will, therefore, probably score 1 or 0. The patient with brainstem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic, score 2. Patients in a coma (item 1a=3) are automatically given a 2 on this item.	0 = **Normal;** no sensory loss. 1 = **Mild-to-moderate sensory loss;** patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick, but patient is aware of being touched. 2 = **Severe to total sensory loss;** patient is not aware of being touched in the face, arm, and leg.	_____
9. Best Language: A great deal of information about comprehension will be obtained during the preceding sections of the examination. For this scale item, the patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet and to read from the attached list of sentences. Comprehension is judged from responses here, as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in a coma (item 1a=3) will automatically score 3 on this item. The examiner must choose a score for the patient with stupor or limited cooperation, but a score of 3 should be used only if the patient is mute and follows no one-step commands.	0 = **No aphasia;** normal. 1 = **Mild-to-moderate aphasia;** some obvious loss of fluency or facility of comprehension, without significant limitation on ideas expressed or form of expression. Reduction of speech and/or comprehension, however, makes conversation about provided materials difficult or impossible. For example, in conversation about provided materials, examiner can identify picture or naming card content from patient's response. 2 = **Severe aphasia;** all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener. Range of information that can be exchanged is limited; listener carries burden of communication. Examiner cannot identify materials provided from patient response. 3 = **Mute, global aphasia;** no usable speech or auditory comprehension.	_____
10. Dysarthria: If patient is thought to be normal, an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barriers to producing speech, the examiner should record the score as untestable (UN), and clearly write an explanation for this choice. Do not tell the patient why he or she is being tested.	0 = **Normal.** 1 = **Mild-to-moderate dysarthria;** patient slurs at least some words and, at worst, can be understood with some difficulty. 2 = **Severe dysarthria;** patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/anarthric. UN = **Intubated** or other physical barrier, explain:_____	_____

Rev 10/1/2003

NIH STROKE SCALE

Patient Identification. ___ ___-___ ___ ___-___ ___ ___

Pt. Date of Birth ___ ___/___ ___/___ ___

Hospital _____(___ ___-___ ___)

Date of Exam ___ ___/___ ___/___ ___

Interval: [ ] Baseline [ ] 2 hours post treatment [ ] 24 hours post onset of symptoms ±20 minutes [ ] 7-10 days
[ ] 3 months [ ] Other _____(___ ___)

11. Extinction and Inattention (formerly Neglect): Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosagnosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable.	0 = **No abnormality.** 1 = **Visual, tactile, auditory, spatial, or personal inattention** or extinction to bilateral simultaneous stimulation in one of the sensory modalities. 2 = **Profound hemi-inattention or extinction to more than one modality;** does not recognize own hand or orients to only one side of space.	_____

_____

_____

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MODIFIED RANKIN SCALE (MRS)

Patient Name: _____
Rater Name: _____
Date: _____

Score	Description
0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

TOTAL (0–6): _____

References

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Scott Med J 1957;2:200-15

Bonita R, Beaglehole R. "Modification of Rankin Scale: Recovery of motor function after stroke."
***Stroke* 1988 Dec;19(12):1497-1500**

Van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. "Interobserver agreement for the assessment of handicap in stroke patients."
***Stroke* 1988;19(5):604-7**

KEYS TO MASTER CHART

IP NO	IN PATIENT REGISTERED NUMBER
NSE	NEURON SPECIFIC ENOLASE
(a)GCS	AT ADMISSION GLASGOW COMA SCALE
(a)NIHSS	AT ADMISSION NATIONAL INSTITUTES OF HEALTH STROKE SCALE
(a) mRS	AT ADMISSION MODIFIED RANKIN SCALE
(a7) GCS	AFTER 7 DAYS GLASGOW COMA SCALE
(a7) NIHSS	AFTER 7 DAYS NATIONAL INSTITUTES OF HEALTH STROKE SCALE
(a7) mRS	AFTER 7DAYS MODIFIED RANKIN SCALE
IV	INFARCT VOLUME
(d) GCS	(a)GCS-(a7)GCS
(d) NIHSS	(a)NIHSS-(a7)NIHSS
(d) mRS	(a)mRS-(a7)mRS

MASTER CHART

STUDY GROUP

sl no	IP no	NAME	AGE	SEX	NSE	(a)GCS	(a)NIHSS	(a)mRS	(a7)GCS	(a7)NIHSS	(a7)mRS	IV	(d)GCS	(d)NIHSS	(d)mRS
1	7668	Basappa	60	MALE	5.998	15	11	5	15	10	5	52.5	0	-1	0
2	10278	Hussan	57	MALE	2.496	12	12	4	15	3	4	14	3	-9	0
3	9364	Jaganath	55	MALE	3.054	7	34	5	7	24	6	3.5	0	-10	1
4	11501	Rudrappa	63	MALE	4.957	15	4	3	10	19	6	31.5	-5	15	3
5	10442	Tippana	75	MALE	2.139	15	5	5	15	5	4	21	0	0	-1
6	28257	gangawa	65	Female	2.202	15	15	4	15	13	4	7	0	-2	0
7	13848	Mallango	50	MALE	2.712	11	19	5	13	17	4	87.5	2	-2	-1
8	10645	harish	48	MALE	1.913	15	1	5	15	0	3	10.5	0	-1	-2
9	26039	suresh	65	MALE	2.78	15	5	4	15	4	3	262.5	0	-1	-1
10	13901	Siddamma	60	Female	4.406	15	12	4	15	6	4	7	0	-6	0
11	6540	Mallapa	65	MALE	2.362	15	2	3	15	0	2	35	0	-2	-1
12	28675	Sumitra	50	Female	2.659	15	5	4	15	4	4	168	0	-1	0
13	14167	Neelaka	45	Female	3.99	15	9	4	15	5	4	7	0	-4	0
14	15294	Yallapa	35	MALE	0.539	3	23	5	10	23	5	490	7	0	0
15	17725	Basappa	60	MALE	3.69	15	12	4	15	12	3	42	0	0	-1
16	15972	Sangayya	64	MALE	2.12	15	2	4	15	0	3	28	0	-2	-1

17	16590	Boramma	80	Female	4.683	7	21	5	9	19	4	21	2	-2	-1
18	7941	Devendra	45	MALE	0	15	0	1	15	0	1	3.5	0	0	0
19	7754	Kallapa	80	MALE	1.126	15	1	1	15	0	1	14	0	-1	0
20	5983	Sattawa	50	Female	0.379	14	20	4	15	13	4	525	1	-7	0
21	13933	Rosulbee	65	Female	2.5	15	0	3	15	0	3	3.5	0	0	0
22	23906	shivabai	80	Female	3.514	15	13	4	15	9	4	28	0	-4	0
23	13369	Kantewwa	70	Female	9.485	15	3	4	15	2	4	34.5	0	-1	0
24	18356	Laxman	60	MALE	7.752	9	13	5	11	11	4	7	2	-2	-1
25	12072	Sulochana	90	Female	4.244	11	22	5	10	22	5	1.4	-1	0	0
26	17265	Ambubai	70	Female	3.17	15	2	2	15	2	1	14	0	0	-1
27	22124	Tippana	60	MALE	5.196	15	2	4	15	2	4	21	0	0	0
28	25403	Maheboob	52	MALE	8.935	3	24	5	10	21	5	254	7	-3	0
29	5134	Ram	55	MALE	7.45	15	1	4	15	0	1	36	0	-1	-3
30	29064	Madivallapa	52	MALE	2.743	15	4	4	15	6	3	140	0	2	-1
31	10897	Neelabai	48	Female	6.975	15	2	3	15	1	2	86	0	-1	-1
32	5753	Gamibai	65	Female	1.836	14	10	5	15	10	4	38.5	1	0	-1
33	11773	Shreemant	75	MALE	1.954	15	8	4	15	7	4	510	0	-1	0
34	20447	Chandrakka	54	MALE	8.6	15	5	4	15	5	4	15.5	0	0	0

35	15459	Mayyapa	60	MALE	2.89	15	6	5	15	6	5	70	0	0	0
36	28257	gangawa	65	Female	10.42	15	15	4	15	13	4	14	0	-2	0
37	20509	sumitra	63	Female	16.78	15	4	4	15	3	4	31.5	0	-1	0
38	10136	shivabai	45	Female	15.31	15	2	4	15	2	3	356	0	0	-1
39	1837	mahade	60	MALE	20.2	15	4	5	15	0	3	56	0	-4	-2
40	2873	mahadevi	65	Female	7.56	3	1	3	4	0	2	56	1	-1	-1
41	695	kallawa	82	Female	9.568	15	20	5	15	20	5	112	0	0	0
42	3099	shrishail	60	MALE	2.56	15	10	5	15	12	5	220.5	0	2	0
43	3362	neelagang	80	Female	5.209	15	12	5	15	14	5	90	0	2	0
44	4386	sidappa	70	MALE	20.23	15	10	5	15	12	5	106	0	2	0
45	7689	muttapa	80	MALE	0.702	15	11	5	5	18	5	35	-10	7	0
46	12571	shankunta	75	Female	4.49	15	9	4	15	7	4	32.5	0	-2	0
47	19783	shankrapp	72	MALE	16.78	15	10	4	15	8	4	3.5	0	-2	0

CONTROL GROUP

sl no	IP no	name	age	sex	NSE	(a)GCS	(a)NIHSS	(a)mRS	(a7)GCS	(a7)NIHSS	(a7)mRS	IV
48	25508	jagadesh	60	MALE	0.245	15	0	0	15	0	0	0
49	28231	nandakum	56	MALE	0.004	15	0	0	15	0	0	0
50	25584	huseini	55	MALE	0.076	15	0	0	15	0	0	0
51	28563	mallangow	63	MALE	0.023	15	0	0	15	0	0	0
52	12350	mahadev	75	MALE	0.087	15	0	0	15	0	0	0
53	28736	sattawa	65	Female	0.005	15	0	0	15	0	0	0
54	24461	sharanu	50	MALE	0	15	0	0	15	0	0	0
55	25489	veerabhad	47	MALE	0.876	15	0	0	15	0	0	0
56	26666	suhgappa	65	MALE	0.046	15	0	0	15	0	0	0
57	27364	yamana	60	Female	0.023	15	0	0	15	0	0	0
58	26512	dhalappa	65	MALE	0.002	15	0	0	15	0	0	0
59	25256	nilawati	52	Female	0.88	15	0	0	15	0	0	0
60	27533	kusanabai	45	Female	0.008	15	0	0	15	0	0	0
61	27523	pandu	35	MALE	0	15	0	0	15	0	0	0
62	28543	kallapa	60	MALE	0	15	0	0	15	0	0	0

63	27405	sangappa	64	MALE	0.34	15	0	0	15	0	0	0
64	28443	laxmibai	78	Female	0.01	15	0	0	15	0	0	0
65	25866	shrishail	46	MALE	0	15	0	0	15	0	0	0
66	29332	ram	80	MALE	0.023	15	0	0	15	0	0	0
67	24071	prema	50	Female	0	15	0	0	15	0	0	0
68	24560	muragaw	65	Female	0.078	15	0	0	15	0	0	0
69	27878	ratnabai	80	Female	0.234	15	0	0	15	0	0	0
70	25336	shivalinga	70	Female	1.768	15	0	0	15	0	0	0
71	27749	tarbasha	60	MALE	0.037	15	0	0	15	0	0	0
72	6829	mallamma	45	Female	0.008	15	0	0	15	0	0	0
73	27873	mosumbe	70	Female	0.001	15	0	0	15	0	0	0
74	27400	shivappa	60	MALE	0.007	15	0	0	15	0	0	0
75	29099	shantosh	52	MALE	0.034	15						
76	25781	bhimasing	55	MALE	0.789	15	0	0	15	0	0	0
77	27075	rajaksab	53	MALE	0	15	0	0	15	0	0	0
78	25965	yallama	48	Female	2.789	15	0	0	15	0	0	0
79	26008	pemala	65	Female	0.98	15	0	0	15	0	0	0

80	25462	tippanna	71	MALE	0	15	0	0	15	0	0	0
81	26233	basangond	47	MALE	0.005	15	0	0	15	0	0	0
82	27903	mallango	60	MALE	0.006	15	0	0	15	0	0	0
83	25944	kamalabai	65	Female	0.044	15	0	0	15	0	0	0
84	24618	laxmibai	63	Female	0.004	15	0	0	15	0	0	0
85	25692	gouramma	45	Female	0.324	15	0	0	15	0	0	0
86	27923	balakrishn	60	MALE	0.003	15	0	0	15	0	0	0
87	25944	KAMALBA	65	Female	0.008	15	0	0	15	0	0	0
88	15909	imambu	82	Female	0.007	15	0	0	15	0	0	0
89	25964	tamanna	60	MALE	0	15	0	0	15	0	0	0
90	19680	nagawwa	70	Female	0.07	15	0	0	15	0	0	0
91	25715	basayya	70	MALE	0	15	0	0	15	0	0	0
92	25657	shivagonda	77	MALE	0.321	15	0	0	15	0	0	0
93	26226	gangabai	75	Female	0.006	15	0	0	15	0	0	0
94	27070	damodar	72	MALE	0.046	15	0	0	15	0	0	0