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Assessment of Serum Neuron Specific Enolase (NSE) and Other Biochemical Parameters in Iraqi Patients with Ischemic Stroke

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ABSTRACT

Background:''Neuron specific enolase'' (NSE) is, a dimeric iso-enzyme of the glycolytic enzyme enolase that is localized mostly in the neurons.

Aim: To assess the association between serum NSE levels with stroke severity.

Methods: The present study has been carried out on a total of 88 subjects, divided into three groups: G1 group, (29) ischemic stroke without hypertension and diabetes mellitus, while G2 group, (29) ischemic stroke with hypertension and diabetes mellitus, and control group (C), (30) healthy persons. Serum concentrations of ''NSE, random sugar, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and electrolytes (Na, Ca, and K)'' for all participants were measured.

Results: The levels of random sugar, urea, ALT, AST, Na, K, Ca, RBCs, MCH, HCT and HGB were significantly increased in the C group compared to the G1 and G2 groups ($p< 0.01$). Moreover, the NSE level was significantly increased in patients (G1 and G2 groups) compared to control group (51.94 ± 14.16 ng/ml, 55.12 ± 10.085 ng/ml and 14.29 ± 1.98 ng/ml respectively). It can be concluded that serum NSE level may be used as an early detection marker of ischemic stroke patients.

Conclusion: Our result verified that NSE serum levels during the early stages of an ischemic stroke can act as a useful marker to anticipate stroke, the intensity and quick functional consequence. Additionally, the various blood pressure factors are each independently linked to a higher risk of stroke in people with diabetes.

Keywords: Ischemic stroke, Diabetes mellitus, Hypertension, Neuron specific enolase.

INTRODUCTION

Stroke is defined as: "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" [(1)]. Three different types of stroke exist: ischemic stroke, transient ischemic attack (a warning or "mini-stroke") and hemorrhagic stroke [(2)].

A localized decrease of blood flow to the brain's tissue as a result of stenotic or blocked cerebral vasculature results in an ischemic stroke (IS). As a result, brain function declines correspondingly [(3)]. There are two main ischemic stroke categories: Thrombotic strokes, which occur when a blood clots that occur in the artery supplying blood to the brain and embolic strokes that occur when a clot forms elsewhere in the body and passes through the blood arteries in the brain [(3, 4)].

The epidemiological research has demonstrated that hypertension and diabetes are common correlated conditions and their concordance is increasing in populations [(5,6)]. Up to 40% or more of diabetes people have hypertension. One of the major stroke risk factors is high blood pressure in the general population. Although some studies, but not all, have identified diabetes or hyperglycemia as an independent stroke risk factor. It been discovered that the independent effect of these conditions on stroke risk is inconsistent. [(7)]. Although there are few research on the combined prognostic impact of hypertension and diabetes on the prevalence of the unknown population's stroke risk whether the elevated stroke danger is caused by each of these two conditions alone or by their combined impact [(5, 8)].

The biomarkers that are used in the diagnosis of stroke, as well as indicators of stroke severity and prognosis, is receiving special attention [(9)]. One such biomarker is Neuron Specific Enolase, an isoenzyme of the glycolytic enzyme enolase that is dimeric, which is mostly expressed in the neuroendocrine system's neurons and cells [(10)]. In cases of ischemic or hemorrhagic stroke neuron specific enolase concentrations can serve as an indication for brain's nerve cells. Immediately following hemorrhagic or ischemic brain injury, cerebrospinal fluid (CSF) and blood show rising NSE levels [(11)]. Following an ischemic stroke and other brain tissue traumas like intracerebral hemorrhage, head injury and subarachnoid hemorrhage, NSE concentrations rise in the CSF. Between 4 and 8 hours after the stroke's start, NSE was first identified [(12)].

The principal characteristics ischemia signals in the CNS include the peak and the ischemia cascade's early buildup, rapid dissemination either by cell-borne transmission or through ischemic tissue into the circulations in close proximity to blood vessels as a response to ischemia occurrences. Also has a few-hour half-life or more and only present throughout ischemia nerve tissue [(13)]. Inside of the initial 96 hours of cerebral infarction and in certain circumstances, the sixth day following an infarction, blood NSE levels reach their peak. The highest serum level of NSE occurs 72 hours following an infarction occurrence. Before irreversible neuronal damage occurs, the elevated serum NSE can

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be diagnosed since it is connected to NSE in neurons [14]. The study's purpose was to assess the relationship between NSE serum levels of stroke severity and to evaluate the real relationship between hypertension and diabetes on the probability of stroke.

MATERIALS AND METHODS

The Subjects

Fifty-eight (58) patients admitted with acute ischemic stroke to the Neurosurgery Hospital and Neuroscience Hospital (Iraq-Baghdad) from February 2021 to May 2021 were included in the study. G1 group included 29 (18 males &11 females) ischemic stroke patients without diabetes mellitus and hypertension, while G2 group included 29 (13 males & 16 females) ischemic stroke patients with diabetes mellitus and hypertension, and control group (C) that included 30 (16 males & 14 females) healthy persons aged between forty and eighty years. Clinical symptoms, physical examination, cranial computed tomography (CT), or magnetic resonance imaging (MRI) were used to diagnose all IS patients.

Exclusion criteria: Patients with renal, hepatic and serious cardiac disorders, a past history of a stroke, hemorrhagic stroke, transient ischemic attack (TIA), recent head injury and CNS tumors.

Five milliliters of venous blood taken with a gel separator in biochemistry tubes were taken from each participant. Centrifuging was done on the samples (at 1500 g for fifteen minutes) following a thirty-minute incubation period. The estimation was done using a part of the acquired serum. Human NSE levels were measured using ELISA method as per the manufacturer's instructions.

Biochemical parameters assay:

Random glucose, calcium, and sodium were determined by Spectrophotometer apparatus (Hettich/Germany), which is based on the intensity of radiation emitted at a wavelength characteristic for a given element [15-17]. Potassium was determined by Reflotron plus device (Roche/ Germany), which used Reflotron test reagent strips parameters [18]. A kit (biomerieux/ France) was used to assess serum urea using an enzymatic procedure (Urease-modified Berthelot reaction) [19]. The colorimetric methodology with deproteinization utilizing a kit (Syrbio/France) was used to determine serum creatinine. [20]. The AST and ALT enzyme activity was estimated utilizing the French company BIOMERIEUX [21, 22]. The CBC was determined by using an auto hematology analyzer that automatically, measures and prints the results [23].

Ethical approval: The study was authorized from The Research Ethics Committee, College of Science Baghdad University. The World Medical Association's Code of Ethics (Declaration of Helsinki) for studies involving humans has been followed in the performance of this work. The patients' or their closest relatives' informed consent was obtained.

Statistical Analysis

Version 26 of the SPSS statistical analysis software was used for statistical analysis purpose of data. Percentages were used to indicate categorical variables, whereas means and standard deviations (mean ± SD) were used to express continuous variables. The data analyzed significant differences between means that were assessed by using one way analysis of variance (ANOVA). Results were considered significant with $p \leq 0.05$ while $P < 0.01$ is highly significant. Receiver operating characteristic (ROC) curve analysis and the correlation coefficient was measured by the Pearson correlation coefficient and was used to separate ischemic stroke patients from controls and to assess the efficacy of the test.

RESULTS

The study included eighty eight subjects who were divided into three groups: the G1group (ischemic stroke without diabetes mellitus and hypertension) (n=29) involved 18 males (62%) and 11 females (37.9%), G2 group (ischemic stroke with diabetes mellitus and hypertension) (n=29) involved 13 males (44.8%) and 16 females (55.1%) and the control group (n=30) that involved 16 males (53.3%) and 14 females (46.7). Table (1) showed the individuals' general anthropometric, clinical, and biochemical characteristics.

Table (1): Baseline clinical characteristics of stroke patients (G1, G2) and healthy control

Groups / Variables	G1	G2	C
N	29	29	30
Gender (M/F)	18/ 11	13 /16	16/14
Family history, n (%)	7 (24.13%)	9 (31.03%)	12 (40%)
Smoker, n (%)	10 (34.48%)	14 (48.27%)	13 (44.82%)
Using of alcohol, n (%)	4 (13.79%)	2 (3.44%)	0
HTN, n (%)	0	29 (100%)	15 (50%)
DM, n (%)	0	29 (100%)	8 (27%)

DM= Diabetes mellitus, HTN= Hypertension

Table (2) illustrated the biochemical parameters for all studied groups. The age showed highly significant increases in G2 group in comparison with G1 and healthy group ($p < 0.01$). Moreover, systolic blood pressure (SBP) demonstrated that there was a highly significant difference in G1 and G2 in comparison with the control group ($p < 0.01$). The results also demonstrated highly significant increases in urea level in G1 group than in the control group ($p < 0.01$). But there was non-significant difference between G1 and G2 groups. However, random sugar in G2 group was significantly greater than in the healthy group ($p < 0.01$), but there was non-significant difference between G1 and G2 groups. Also, WBC, RBC and MCH showed that there were a highly significant differences in G2 and control groups when compared to G1 group ($p < 0.01$). By comparing the means of Na, K and Ca, there was a significant increase in control group compared to G1, G2 groups. Serum NSE Mean values was significantly higher in G1 and G2 groups compared to control group. While, NSE mean level of G2 group showed a significant increase when compared to G1 group as shown in table (2).

Table (2): Mean values and ± SD of Anthropometric and Biochemical parameters in control and patients groups

Groups / variables	G1	G2	C	*P Value*
N	29	29	30	
Age (year) Mean± SD	54.793± 8.474	70.241 ±5.356$^{**b, c}$	60.533 ±11.309	P<0.01
BMI (Kg / m^2)	29.860±3.858	29.106 ±4.439	28.085±2.683	0.19012
SBP (mmHg)	148.586±27.314	147.310±28.613^{**b}	116.833±20.987	P<0.01
DBP (mmHg)	86.275±15.804	80.344± 24.776	80.166±8.757	0.326107
Urea	44.724±16.195^{**a}	40.551±13.873$^{**b, c}$	28.700±6.412	P<0.01
Creatinine	0.844±0.227	0.882±0.217	0.7850±0.107	0.14670
Sugar	151.916±45.358^{**a}	165.736±40.944$^{**b, c}$	85.809±15.157	P<0.01
WBC× 10^9/ L	11.263 ±2.703^{**a}	12.138±2.167^{**b}	7.566±2.128	P<0.01
RBC × 10^{12}/ L	4.102±0.674^{**a}	3.842±0.847^{**bc}	4.739±0.577	P<0.01
MCH pg	30.082±2.615^{**a}	31.288±1.639^{**b}	25.293±3.246	P<0.01
MCV (fL)	83.586±8.705	84.989±5.776	82.940±11.404	0.670668
HCT %	39.148±6.892^{*a}	40.033±6.150^{*b}	44.130±2.652	0.001725
HGB (g/dl)	10.072±0.958^{**a}	9.982±1.735^{**b}	12.114±1.171	P<0.01
PLT × 10^3/ μl	281.931±29.753	283.434±28.233	248.633±27.748	0.164976
LYMPH × 10^9/ L	1.930±0.11^{*a}	2.957±0.300$^{*b, c}$	1.769±0.178	0.047081
RDW %	14.452±1.913	14.296±1.7268	13.683±1.353	0.181869
ALT (U/L)	24.672± 4.461^{*a}	21.546±5.572$^{*b, c}$	26.766±6.785	0.0181
AST(U/L)	27.658±5.625^{*a}	22.448±3.488$^{*b, c}$	25.500±5.556	0.0206
Na (mEq/L)	135.655±4.134^{**a}	133.127±6.742$^{**b, c}$	139.666±2.682	P<001
K (mEq/L)	3.675±0.580^{*a}	3.779±0.395^{*b}	4.100±0.419	0.002
Ca (mEq/L)	1.416±0.330^{**a}	1.468±0.431^{**b}	2.243±0.207	P<0.01
NSE (ng/ml)	51.949±14.167^{**a}	55.124±10.085^{**bc}	14.291±1.988	P<0.01

***P* < 0.05 Significant, ** *p*< 0.01 is highly significant, a illustrates to the significant differences between G1 and control, b illustrates to the significant differences between G2 and control, c illustrates to the significant differences between G1 and G2. (SBP)=** ''Systolic blood pressure''**, (DBP)=** ''Diastolic Blood Pressure'', **(WBC)=** White Blood Cell**, (RBC**)= Red Blood Cell**), (MCV)=** Mean Corpuscular Volume **, (MCH) =** Mean Corpuscular Hemoglobin**, (HCT)=** Hematocrit**, (HGB)=** Hemoglobin**, (PLT)=** Platelet, **(LYMPH)=** Lymphocyte**, (RDW)=** Red Blood Cell Distribution Width**, (AST)=** Aspartate Aminotransferase**, (ALT)=** Alanine Aminotransferase**, (NSE)=** Neuron Specific Enolase.

Table (3) showed the Pearson correlation coefficient of serum NSE with the other parameters in G1 and G2 groups. Based on the result, G1 group's serum NSE had significant positive correlation with BMI (*P* = 0.008), (R= 0.4793), while G2 group, no significant correlation occurred between NSE and other parameters.

Table (3): Person Correlation between concentration of NSE and biochemical parameters in G1 and G2 of stroke patients

Parameter	Serum NSE					
	G1 Group (N=29)			G2 Group (N=29)		
	R	*P*	Sig.	R	*P*	Sig.
Age	0.11913	0.5382	N S	0.1672	0.385	N S
BMI	0.4793	0.0085	H S	0.0480	0.808	N S
SBP	-0.2578	0.1768	N S	-0.228	0.232	N S
DBP	-0.0870	0.6533	N S	0.0229	0.905	N S
Urea	0.04563	0.8141	N S	-0.142	0.461	N S
Creatinine	0.29157	0.1248	N S	0.0316	0.870	N S
Sugar	0.32058	0.0899	N S	0.2721	0.153	N S
Na	-0.0318	0.8697	N S	-0.300	0.113	N S
K	0.34576	0.0661	N S	-0.005	0.976	N S
Ca	0.00444	0.9817	N S	-0.193	0.315	N S
WBC	0.21143	0.2708	N S	-0.211	0.270	N S
RBC	0.34770	0.0645	N S	-0.257	0.176	N S
MCH	-0.0186	0.9233	N S	-0.137	0.476	N S
MCV	0.19719	0.3052	N S	-0.155	0.419	N S
HCT	0.24432	0.2014	N S	-0.225	0.240	N S
HGB	0.23345	0.2229	NS	-0.221	0.248	N S
PLT	-0.2173	0.2574	NS	-0.286	0.131	N S
LYMPH	-0.2371	0.2154	NS	0.135	0.484	N S
RDW	-0.1178	0.5427	NS	0.0728	0.707	N S
ALT	0.10293	0.5951	N S	0.2466	0.197	N S
AST	0.048778	0.8016	N S	0.194	0.311	N S

H S: P < 0.001, S: P < 0.05, N S: P > 0.05

ROC curve for (NSE) level and groups of patients: ROC analysis was utilized to identify ischemic stroke groups (G1 & G2) and to distinguish these patients from the danger group. Table (4) and figures (1 & 2) illustrated that NSE had area under the curve of 1 with a significance of P < 0.01 among ischemic stroke patients with and without HTN and DM.

Table (4): Area under the Curve (AUC) for the investigated parameter of G1, and G2

Parameter	AUC	*P*- value	95% Confidence Interval (CI)	
			(Lower Bound)	(Upper Bound)
NSE (ng/ml)	1	<0.01	1	**1**

(AUC)= Area under the curve

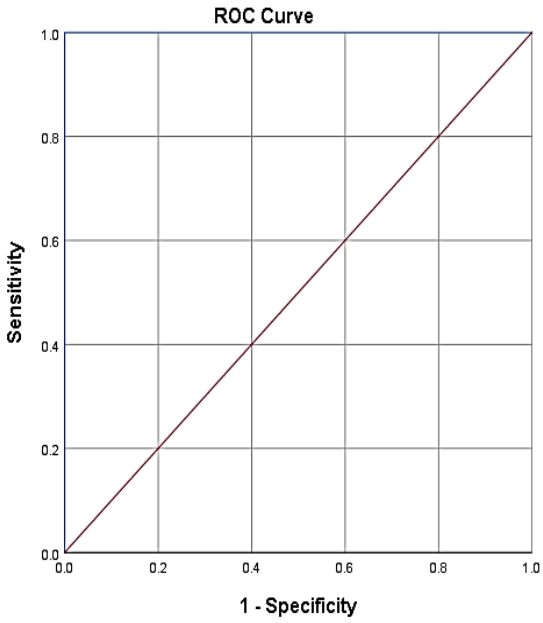
The cut off values of NSE for G1 and G2 groups were 24.64 ng/ml and 28.423 ng/ml respectively. The percentage of sensitivity, specificity, PP and NP values of NSE in G1 and G2 groups was 100% for all (Tables 5 & 6).

Table (5): Cut off points, sensitivity, Specificity, Positive predictive, Negative predictive for the investigated parameter of G1 and control

Parameter	Cut off point	% Sensitivity	% Specificity	Positive predictive	Negative predictive
NSE (ng/ml)	24.643	%100	%100	100	100

Table (6): Cut off points, sensitivity, Specificity, Positive predictive, Negative predictive for the investigated parameter of G2 and control

Parameter	Cut off point	% Sensitivity	% Specificity	Positive predictive	Negative predictive
NSE (ng/ml)	28.423	%100	%100	100	100



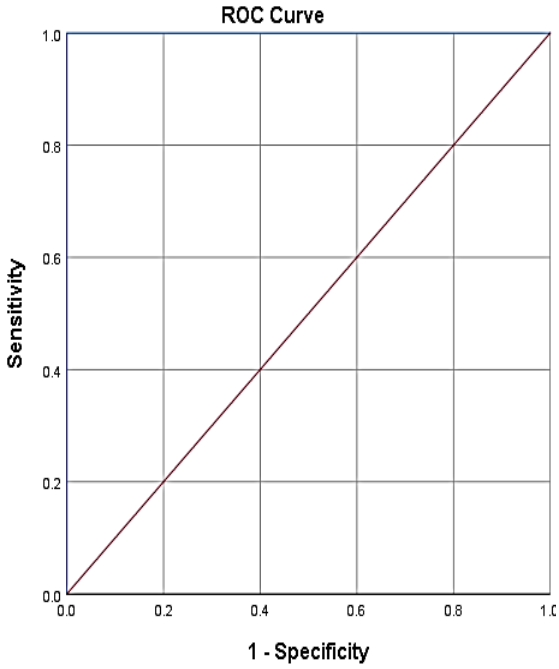


Figure (1): ROC curve for NSE of G1 group
Figure (2): ROC curve for NSE of G2 group

DISCUSSION

NSE is an isoenzyme of the glycolytic enzyme enolase that is dimeric and having a molecular weight of around 80,000 Da. The neuroendocrine system's neurons and cells are the primary locations of NSE. Since NSE is mostly present in neurons, neurological illnesses often cause an increase in NSE levels in the serum or CSF [24]. The present research evaluates the NSE's role as a biomarker to detect the brain parenchymal damage in ischemic stroke and thereby track the course of the disease. Instead of the CSF, highly sensitive brain indicators must be able to be detected in blood.

The study was carried out on 58 patients (G1, 29) and with ischemic G2 (29) of stroke, evaluated the joint association between hypertension and DM incidence of stroke. The study's findings showed that patients with higher levels of serum NSE included ischemic stroke G1 and G2 than in the healthy individuals ($P < 0.01$). This is in line with research conducted by **Bharosay *et al.*** [25] and **Padalkar *et al*.** [26]. As NSE was found inside of neuronal cells' cytoplasm, its inclusion in the serum at significantly high levels imply neuronal injury. This is in line with **Mohammed** [27], who noted that endothelial cells loss after a stroke affects the blood brain barrier (BBB), and through it, the cytoplasmic materials were released from the injured brain regions disseminate. In the present study, an increased level of NSE is thought to be associated to cerebral vascular stroke. NSE level rises during ischemia because of hypoxia, brain ischemia, convulsion and injury. The Blood-brain barrier is degradation and astroglial breakdown leads to NSE leaking. Additionally, this investigation discovered that NSE levels in the blood had a significance ($P < 0.01$) with the NIHSS scores in ischemic stroke.

A rise in serum NSE levels is influenced by a number of factors, including lesion location and metabolic syndrome [28]. NSE concentrations are higher in patients with metabolic syndrome, according to research by **Ospanov *et al.*** [29]. Hypertension, insulin resistance, obesity, and dyslipidemia are all symptoms of the metabolic syndrome that leads to a persistent inflammatory condition and ongoing cytokine circulation TNF, which tumor-necrosis factor is one of them. This syndrome results in aberrant blood-brain barrier permeability and endothelial dysfunction. The brain's tiny arteries are affected by hypertension and dyslipidemia, which disrupts metabolic processes in the surrounding neurons and astrocytes. Chronic hypoxia is a result of atherosclerosis. Increased glutamate release from TNF- results in interruption and excitotoxicity of neuronal flow. In addition, hypoxia can result in impaired energy metabolism, mitochondrial dysfunction, lipid peroxidase as well as neuronal apoptosis. This damages of neurons, release NSE into the bloodstream as a result [30,31, 32].

Ischemic stroke clinical outcomes are affected by diverse factors including being overweight, having high blood pressure, smoking in the past, having a history of hypertension, and having a history of dyslipidemia [33]. In this study, these factors were taken into account. Additionally, the treatment provided to each patient differently can have an influence, thus, the clinical results for the patients were not the same. Because of the nature of stroke, endothelial cell death results in damage to the brain-blood barrier, in addition to the blood-brain barrier being permeable to the cytosolic component produced from damaged brain tissue. NSE levels were related to stroke-related brain parenchymal damage and secondary neuronal damage mechanisms brought on by edema and elevated

intracranial pressure [25]. As mentioned by **Haquee *et al.*** [34], elevated levels of NSE can have harmful influences by inducing apoptosis in neuronal cells and increasing the release of proinflammatory cytokines. Actin remodeling, inflammatory glial cell proliferation, and extracellular matrix breakdown can all be accelerated by increased NSE, therefore influencing the movement of activated microglia and macrophages to the damaged location and increased loss of neuronal cells. In the current study, the effectiveness of serum NSE in the detection of ischemic stroke was analyzed. The efficiency of serum NSE in ischemic stroke detection estimated in G1 and G2 groups (24.643 ng/ml and 28.423 ng/ml respectively) with specificity of 100%, sensitivity of 100% and the area under curve for NSE was (1). These findings are consistent with **Philipp *et al.*** [35] research. They found in a single examination of NSE a sensitivity of 89%. In addition, **AL- Rawy *et al.*** [36] showed that serum NSE's area under curve had a significantly greater value (0.960) in comparison with NSE in the saliva (0.82) and the most effective cut off for serum NSE with greatest diagnostic accuracy (90%) ≥13.1µg/L. This threshold for cut-off had a reasonable sensitivity of 100%. **Padalakari *et al.*** [26] discovered that the cut off point maximizing the specificity and sensitivity had been found to be 40 ng/ml with a sensitivity of 87.10% and area under ROC curve for NSE of 0.84. A greater degree of disability and a worsening of the neurological condition were related to higher serum levels of NSE.

CONCLUSION

Our result verified that NSE serum levels during the early stages of an ischemic stroke can act as a useful marker to anticipate stroke and the intensity and quick functional consequence. Additionally, the various blood pressure factors are each independently linked to a higher risk of stroke in people with diabetes.

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