Determination and Diagnostic Significance of Serum Neuron Specific Enolase (NSE) Levels in Patients after Cardiac Arrest and Cardiac Dysfunctions

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Abstract: Present study describes the determination and evaluation of diagnostic significance of Neuron Specific Enolase (NSE) in patients with cardiac arrest and related cardiac dysfunction. **Objectives:** To examine the diagnostic and prognostic value of serum NSE for early prediction of adverse clinical outcome in patients after cardiac arrest. **Study Designs**. *Place and Duration of the study.* Department of Biochemistry Laboratory, Liaquat National Hospital, Karachi from June 2006 to December 2006. *Patients and Methods*: A total of 72 patients, cumulative mean age 56.21 ± 5.90, (52 males, 20 females) brought to the Emergency Department of LNH with cardiac arrest, dysfunction and abnormalities, were included in the study. Each of these patients was successfully resuscitated and shifted to either ICU or CCU. Blood samples were taken to compare levels of NSE along with other diagnostically significant markers such as AST, LDH, CK and CKMB iso-enzymes. **Results:** Increasing levels of NSE significantly ($P<0.01$) correlated with the increasing value of CK and CKMB. Moreover, high AST levels also show borderline significance with high NSE level, when gender-wise independent t-test for equality of variance was performed. The results depict further diagnostic importance of NSE, at par with three out of four biochemical cardiac markers (CK, CKMB, AST except LDH). **Conclusion:** In present study, estimated NSE levels, determined in a group of males and females patients suffering from cardiac arrest or related cardiac dysfunction, was found to be a valuable diagnostic tool, statistically evaluated to be a par with the other commonly used diagnostically important cardiac enzymatic markers, CK and CKMB (inclusive of AST when correlated cumulatively).

Key Words: Neuron Specific Enolase, Cardiac Arrest, Diagnostic Significance.

INTRODUCTION

Several advances have came into existence regarding cardiac pulmonary managements, however, morbidity and mortality associated with cardiac arrest remain extremely high [1]. The prognosis in this specified condition is also not very promising as several patients ultimately suffers from mild to moderate disability to persistent vegetative state, which are termed as unfavorable or adverse clinical outcome [1]. It is reported that in 80% cases of sudden death survivors, a comatose state prevails for various lengths of time, and a full neurological recovery is rare [1, 2]. It has been postulated that an irreversible anoxic brain damage might have occurred soon after cardiac arrest. Therefore suggestions have been made to facilitate evaluation of cardiac arrest patients, for finding out an accurate prognostic tool which may contribute fully and eliminate major ethical and economic consequences [1].

Presently, prognosis in cases of cardiac arrest and adverse outcome are based on several clinical, neuro-imaging and electrophysiological methods [1, 3-6], which some times difficult to administer and manage. In this regard bio-chemical markers, in comparison, are examined to be a low cost-alternative that may be more suitable both economically and management-wise. Neuron-specific enolase (NSE) is a known marker of ischemic brain damage and already been evaluated in traumatic brain injury [7], stroke [8] and anoxic encephalopathy after cardiac arrest [9, 10]. NSE, the neuronal form of the glycolytic enzyme enolase, is found almost exclusively in neurons and cells of neuroendocrine origin [1]. It is a di-meric form compounded of two subunits that converts 2-phosphoglycerate into phosphoenolpyruvate, measurable in blood and cerebrospinal fluid [11]. There are several studies carried out on the prognostic value of NSE in patients surviving in-hospital cardiac arrest [1], post-anoxic coma [12], long-term outcome [13] out of hospital cardiac arrest [14] and cardiac surgery [15]. The present study is based on determination and evaluation of diagnostic importance of NSE in patients with cardiac arrest and/or related cardiac dysfunction.

MATERIALS AND METHODS

The study prospectively evaluated 72 patients (Males 52, Females 20) who came to Emergency Ward (ER) of the hospital in the state of cardiac arrest in the period from June 2006 to December 2006 at Liaquat National Hospital, a tertiary-care hospital of Karachi. The patients', mean age 56.21 ± 5.90 yrs, who were included, were successfully resuscitated after coming to ER for cardiac arrest or related

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0250-5363/09

cardiac dysfunction and exhibiting pulseless ventricular tachycardia, pulseless electrical activity and asystole or premature ventricular contraction, ventricular tachycardia, arterial flutter, ST segment evaluation and development of abnormal Q-waves. The excluded patients were under the age of 25 years and those presenting drug intoxication, accidental or therapeutic hypothermia, those with neoplastic diseases known to increase NSE levels (stroke ischemic and/or hemorrhagic) or traumatic brain injury and patients subjected to extracorporeal circulation.

Procedure

Protocol of Rech *et al.,* (2006) was followed. Blood sampling took place for NSE measurement between 12 and 36 hours after cardiac arrest. Blood samples were withdrawn by peripheral vein puncture and centrifuged for 10 minutes at 2500 rotations per minutes. Serum (1 ml) was frozen and stored at -86^{o}C. Hemolyzed sample were considered lost. NSE measurements were performed with an electrochemi-luminescence immunoassay (ECLIA), using a sandwich technique, in duplicate, with NSE kits (Roche, Diagnostic, Basil) and the Elecsys 2010 analyzer (Roche Diagnostics, Basil). NSE measurements were also performed in twenty control individuals. Values will be considered normal if is it less than the normal reference value of 1.3 ng/ml (Roche Diagnostics, Basil). Other parameters that were routinely performed were AST, LDH, CK, CKMB on Hitachi 912 (Roche Diagnostic, Basil). Normal reference ranges of AST, LDH, CK and CKMB are, upto 40 U/L, 230-460 IU/L, upto 270 U/L and < 25 U/L, respectively.

Statistical Analysis

Continuous data are presented as means and SD, and nonparametric data as medians range. Student's t test and the Pearson's correlation (2 tailed), were used to compare the data. The discriminative power of NSE to predict an adverse clinical resultant or outcome, such as coma or cardiac failure was determined by analysis of all cardiac parameters (AST, LDH, CK, CKMB). The significance level was set at $p < 0.05$ and $P < 0.01$. Statistical analysis was performed with statistical package for the Social Sciences (SPSS) version 15.

RESULTS

All 72 patients included were grouped according to gender as 52 males and 20 females and were brought to ER with complaints of suspected cardiac arrest and related cardiac dysfunction. During the whole study period upto the end, only 20 females (as compared to 52 males) were brought to E/R with cardiac abnormalities, filling to our inclusion criteria and thus included in the study. Table **1** shows NSE levels measured cumulatively in males and females as a single group as well as individual gender-wise. Moreover NSE levels were measured between 10 to 15 hours after admittance of the patients in ER. Cumulative NSE mean level of all patients was 34.38 ng/ml ± 1.3 with median of 33.0 ng/ml (minimum 18.0 ng/ml; maximum 59.0 ng/ml) (Table **1A**). NSE estimated individually gender wise shows mean level of 35.96 ng/ml ± 1.5 with median of 33.0 ng/ml (Table **1B**). All patients with higher NSE values > 30.0 ng/ml (normal reference value < 3.0 ng/ml) showed equally high

Table 1A. Statistical Analysis of Biochemical Markers per Frequencies in Cumulative Group of both Males (52) and Females (20) Patients

Parameters	AST (IU/L)	LDH (IU/L)	CPK (IU/L)	CKMB (IU/L)	NSE (ng/ml)
N	72	72	72	72	72
Mean	104.04	688.90	632.31	39.40	34.38
± Std Error of Mean	± 14.702	± 18.072	± 22.849	± 1.910	± 1.307

Table 1B. Statistical Analysis of Biochemical Markers per Frequencies for Individual Gender-Wise Group [Males= 52; Females= 20]

Parameters	Gender	N	Mean	± Std. Deviation	± Std. Error Mean
AST	MALES FEMALES	52 20	116.46 71.75	45.039 13.022	20.113 2.912
LDH	MALES FEMALES	52 20	686.79 694.40	156.248 149.334	21.668 33.392
CPK	MALES FEMALES	52 20	653.56 577.05	206.415 147.156	28.625 32.905
CKMB	MALES FEMALES	52 20	39.58 38.95	14.409 20.592	1.998 4.605
NSE	MALES FEMALES	52 20	35.96 30.25	11.348 9.430	1.574 2.109

Units=IU/l for AST, LDH.CPK, CKMB; NSE=ng/ml.

CK and CKMB levels with coherent clinical and cardiac characteristics of moderate to severe potency, where those with NSE levels < 30.0 ng/ml depicts milder clinical signs and symptoms and subsequent mild to moderate levels f CK and CKMB. Interestingly, when CKMB level estimated in patients were compared with NSE (Pearson's correlation, two tailed, $p < 0.05$), a significant correlation was noted ($p < 0.04$) for the group (Table **2**). Similarly, CK comparison with NSE also showed significant correlation ($P < 0.03$) for all 72 patients. Individual gender-wise (Pearson's correlation $P < 0.05$) estimation of NSE with CKMB and CK in males ($P< 0.034$; $P < 0.04$, respectively) (Table **3A**) and females ($P< 0.043$; $P< 0.034$, respectively) (Table **3B**) also showed mild to moderate significance. Such significant correlation of raised NSE levels with CK and CKMB depicts its diagnostic importance in cardiac problems as both CK and CKMB raised values were directly linked with MI, cardiac arrest and related cardiac dysfunction.

Correlation of NSE with AST and LDH were found to be non-significant in all estimations in cumulative group as per Pearson's correlation estimations. However when cumulative-independent sample t-test was performed as per Levene's test for equality of variance, AST and NSE values were statistically significant (Table **4**). The diagnostic significance of NSE in comparison with CK and CKMB was also estimated through regression-correlation graph. NSE showed moderate correlation linearity with CK ($R^2 = 0.119$) and CKMB ($R^2 = 0.114$) in all patients evaluated, where as AST and LDH did not ($R^2 = 0.004$ and $R^2 = 0.015$, respectively), Figs. **1-4**.

DISCUSSION

In the present study, NSE levels, estimated in males and females 10-15 hours after the occurrence of MI, cardiac arrest or related cardiac dysfunction, was found to be a significant diagnostic tool, statistically evaluated to be at par with the other diagnostically important biochemical enzymatic markers CK and CKMB, the two tests of choice in cardiac abnormalities. Previous and recent studies reported the predictive and prognostic importance of NSE in cardiac arrest patients [12-14, 16-19]. It is also reported that NSE levels remain significantly higher after 24 and 48 hrs in patients who didn't regain consciousness even after cardiac-pulmonary resuscitation as compared to the patients who regained consciousness [12]. Moreover, it is also noted that patients who underwent cardiac arrest, and exhibited 10 fold higher NSE concentrations than normal, depicts persistent coma [17]. Similarly, serum NSE levels greater than or equal to 65 ng/ml in non-traumatic cardiac arrest patients suggest increased risk of death or persistent vegetative state [18]. Furthermore, estimation of NSE level was also reported to be

Table 2. Pearson's Correlation Analysis of NSE with other Diagnostically Important Cardiac Biomarkers in Cumulative Group of 72 Patients [Males 52; Females = 20]

Test	Statistical Analysis	CKMB IU/L	AST IU/L	LDH IU/L	CPK IU/L
NSE (ng/ml)	Pearson's Correlation	.337 (**)	0.060	0.121	.344 (**)
	Sig. (2-tailed)	.004	.615	.310	0.003

** Correlation is significant at the 0.01 level (2-tailed).

Table 3A. Pearson's Correlation Analysis of NSE with other Diagnostically Important Cardiac Biomarkers in the Individual Group of 52 Male Patients

Test	Statistical Analysis	CKMB IU/L	AST IU/L	LDH IU/L	CPK IU/L
NSE (ng/ml)	Pearson's Correlation	.295 (**)	0.021	0.066	.286 (**)
	Sig. (2-tailed)	.034	.881	.640	0.040

** Correlation is significant at the 0.05 level (2-tailed).

Table 3B. Pearson's Correlation Analysis of NSE with other Diagnostically Important Cardiac Biomarkers in the Individual Group of 20 Female Patients

Test	Statistical Analysis	CKMB IU/L	AST IU/L	LDH IU/L	CPK IU/L
NSE (ng/ml)	Pearson's Correlation	.486 (**)	0.187	0.347	.457 (**)
	Sig. (2-tailed)	.030	.429	.134	0.043

** Correlation is significant at the 0.05 level (2-tailed).

Table 4. Levene's Test for Equality of Variances Cumulative Group of Cardiac Dysfunction Patients (Males= 52, Female=20).

Tests	Sig.(2-Tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
	Lower	Upper	Lower	Upper	Lower
AST(IU/L)	.032(**)	44.712	20.323	3.950	85.473
LDH(IU/L)	.849	-7.612	39.806	-88.343	73.120
CPK(IU/L)	0.086	76.508	43.613	-11.167	164.183
CKMB(IU/L)	.902	.627	5.019	-9.682	10.936
NSE(ng/ml)	.036(**)	.5712	.2631	.0399	1.1024

**Correlation is significant at the 0.05 level (2-tailed).

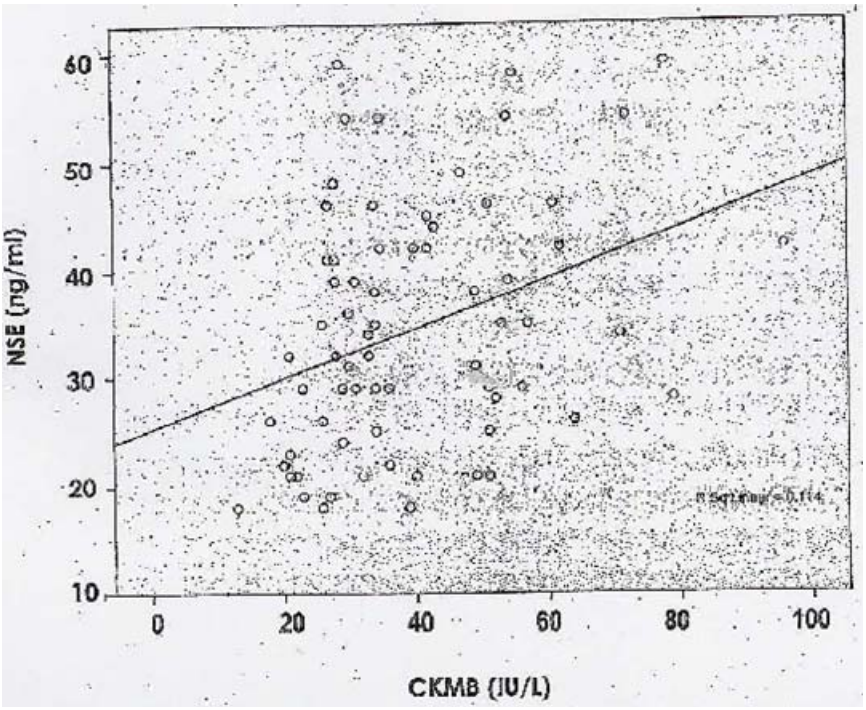

Fig. (1). Regression graph of NSE vs. CKMB in cumulative group of cardiac dysfunction patients (Male = 52, Female = 20).

effective prognostic indicator, along with CKMB in hypoxic brain injury after cardiac arrest [20]. In our study, collective as well group (gender-wise) evaluation of NSE showed similar significant level of diagnostic correlation with clinical characteristics. It has also shown significant correlation ($P < 0.05$) with the established cardiac arrest markers of CK and CKMB, in all patients. NSE estimation and resultant increased level of it, in cardiac arrest patients also predicts long-term outcome in these patients along with S-100 protein, an established biochemical marker of CNS injury [13]. It was asserted that the prognostic value of brain damage marker was comparable with that of traditional clinical parameters [13]. High levels of NSE predict a poor outcome, according to the GOS (Glasgow Outcome Scale) and GCS (Glasgow Coma Scale) [1, 13, 18]. Moreover, increased NSE levels along with S-100 also reported to provide additional information, when estimated to predict cognitive dysfunction after resuscitation from out of hospital cardiac arrest [15].

In present study, increasing level of NSE (18-59 ng/ml; mean 34.38 ngml) also correlated with the increasing value of CK (134-997 IU/L, mean 632.31 IU/L) and CKMB (13-96 IU/L; 39.40 IU/L), both cumulatively and gender-wise, thus suggesting its diagnostic importance in cardiac abnormality and therefore asserting a poor outcome after cardiac arrest or dysfunction. Interestingly, high AST levels (51-664 IU/L; mean 104 IU/L) also depicts borderline significance with high NSE level, when cumulative independent t-test for equality of variance was performed as per Levene's test. This, therefore, depicts a much farther diagnostic importance

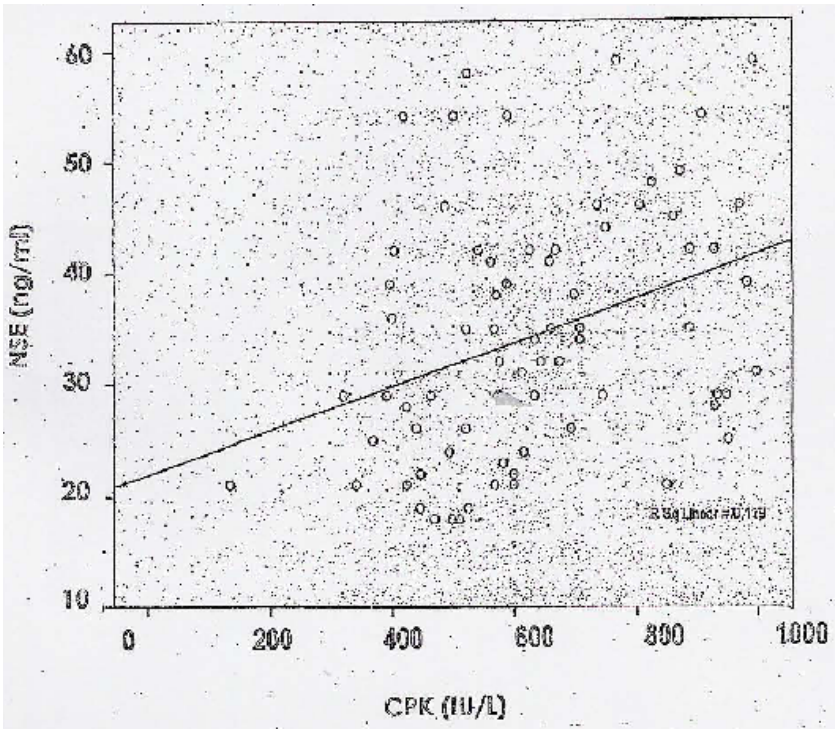

Fig. (2). Regression graph of NSE vs. CK in cumulative group of cardiac dysfunction patients (Male = 52, Female = 20).

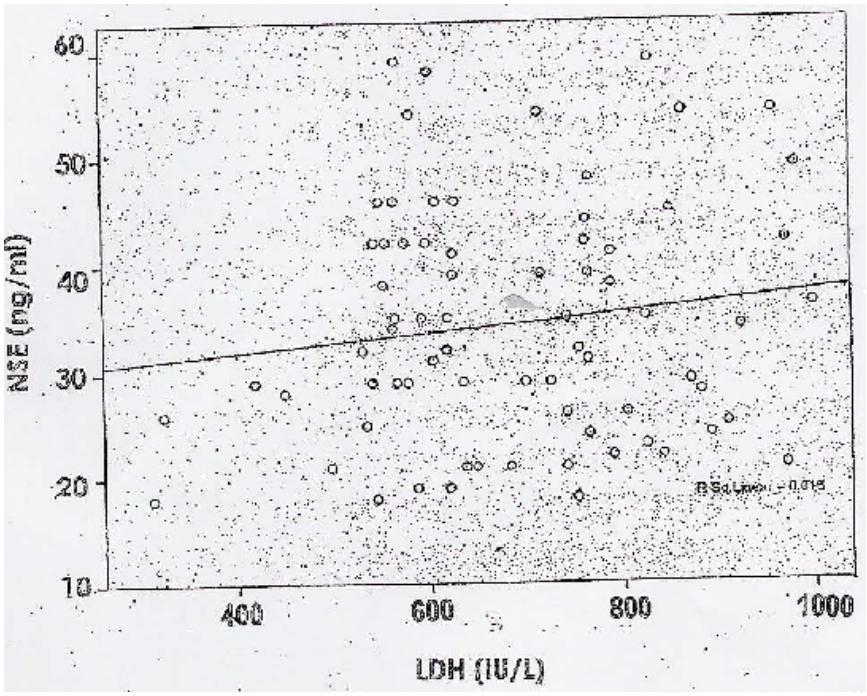

Fig. (3). Regression graph of NSE vs. LDH in cumulative group of cardiac dysfunction patients (Male = 52, Female = 20).

of NSE, at par with three out of four biochemical cardiac markers, i.e. CK, CKMB, AST except LDH.

It was reported that the decreasing NSE values (< 25.0 μg/l) at 6 months intervals in cardiac arrest patients, treated with hypothermia is associated with good outcome ($P < 0.005$), recovery of consciousness ($P < 0.001$) and survival for atleast 6 months after cardiac arrest ($P< 0.012$) [16]. Examination of the prognostic value of NSE for early

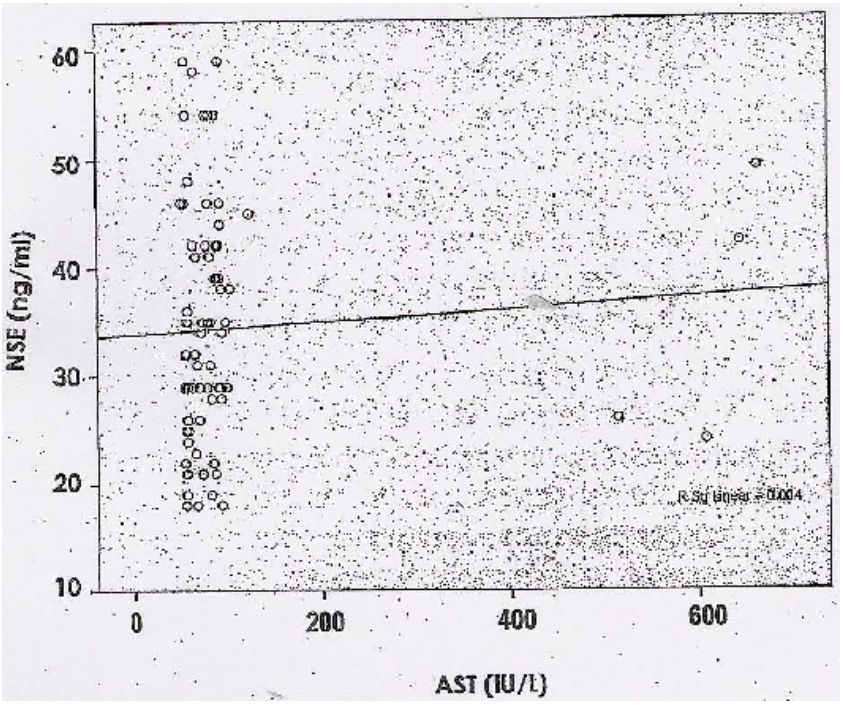

Fig. (2). Regression graph of NSE vs. AST in cumulative group of cardiac dysfunction patients (Male = 52, Female = 20).

prediction of outcome in patients at risk for anoxic encephalopathy after cardiac arrest, also results in high specificity and a positive predictive value [17]. It was suggested that patients who have been resuscitated after cardiac arrest, high levels of NSE predicts persistence coma with high specificity [17]. The combination of GCS and serum value of NSE greater than or equal to a specific higher value of 10 to 20 folds than normal predicts increased risk of death and persistent vegetative state [13]. It was conclude that combined evaluation of GCS with neuro-proteins NSE and S-100 protein at 72 hr after CPR (cardiac pulmonary resuscitation) permits more reliable prediction of outcome in post arrest coma patients [13].

In conclusion, present study represents estimated NSE levels, determined in a group of male and female patients suffering from cardiac arrest or related cardiac dysfunction, that was found to be a significant diagnostic tool, statistically evaluated to be at par with the diagnostically important CK and CKMB (inclusive of AST when correlated cumulatively), the two tests of choice in cardiac abnormalities.

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