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EVALUATION OF NEURON SPECIFIC ENOLASE (NSE) LEVELS IN CHILDREN WITH BACTERIAL AND VIRAL MENINGITIS

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ABSTRACT

The assessment of neuronal markers such as neuron specific enolase (NSE), which enables the diagnosis of meningitis infections and its severity as well as possible development of sub-clinical lesions, could be beneficial and clinically useful. The present study describes the determination of NSE level in the CSF of infants and children after meningitis of both bacterial and viral origins. Fifty eight (58) eligible children between the ages of 1 day and 3 years were selected depending upon history and clinical picture. Infants were divided into gram +ve, gram –ve and viral meningitis groups. CSF and blood samples were collected from all selected patients as part of their routine evaluation for bacterial sepsis and Cerebrospinal fluid (CSF) was analyzed for cell count, protein, and glucose concentrations, NSE and cultures for bacteria and viruses as per standard procedures. Results showed marked elevation in NSE concentrations of CSF in all meningitis groups irrespective of either viral or bacterial origin when compared with control group of non-infected patients (NSE = 1.69 ± 0.91 ng/ml). However, mild to moderate level of elevation was also observed within the meningitis group of viral (NSE = 20.19 ± 2.02 ng/ml) versus *Nesseria meningitis* group (NSE = 50.50 ± 8.96 ng/ml); viral versus *Escherichia coli* (NSE = 35.80 ±6.10 ng/ml) and viral versus *Streptococcus pyogens* group (NSE = 23.75 ± 6.20 ng/ml). The result of NSE being significantly elevated in meningitis patients from all etiological groups supported the hypothesis that meningitis infections and infiltration cause neuronal and neuro-physiological alterations and damages thus causing increase in NSE concentrations.

Key Words: neuron specific enolase (NSE), Vial and bacterial meningeal infections (meningitis).

INTRODUCTION

Meningeal infection (meningitis) in infants and children, both of viral and bacterial origin, is one of the major factors associated with perinatal brain damage (Gazzolo *et al.,* 2004; Nelson and Ellenberg, 1984; Nelson and Grether, 1997, 1998; Yoon *et al.,* 2000). Although curable and monitored well, early stages of meningitis are critical because of the possibility of onset of brain damage at a sub-clinical stage when ultrasound assessment is still silent or un-conclusive (Dammann and Leviton, 1997; Duncan *et al.,* 2002). Laboratory assessment of meningitis is based on chemical analysis of cerebrospinal fluid (CSF) and investigating the causative organisms (bacteria or virus). However assessing and evaluating the cases at high risk is still limited to a few diagnostic investigations (Gazzolo *et al.,* 2004). The measurement of brain markers such as neuron specific enolase (NSE), that would be able to diagnose the severity of meningitis infections and possible occurrence of sub-clinical lesions (Gazzolo *et al.,* 2004), could be beneficial and clinically useful. Several studies have shown that CSF NSE yields a reliable estimate of the severity of neuronal injury as well as clinical outcome of patients with serious clinical manifestations such as in cases of meningitis (Gazzolo *et al.,* 2004; Lima *et al.,* 2004), stroke (Hay *et al.,* 1984), head injury (Persson *et al.,* 1987), anoxic encephalopathy (Roine *et al.,* 1989), encephalitis (Studahl *et al.,* 2000), brain metastasis (Royds *et al.,* 1981), and status epilepticus (Correale *et al.,* 1998). Unlike the other markers of brain injury that have been studied in children (Lima *et al.,* 2004), NSE is brain-specific, and its presence in the CSF is specific for neuronal death. For present study, we hypothesized that NSE level would be increased in the CSF of infants and children after meningitis of both bacterial and viral origin versus a comparison group.

MATERIALS AND METHODS

Study design. Protocol of Lau (2008) and Lau *et al.,* (2008) was followed for proper completion of procedures. The preset study was prospective, cohort conducted at the Biochemistry laboratory services, Liaquat National Hospital, Karachi, and Department of Pathology, Government Lyari General Hospital, Karachi, between January 2005 and November 2006. Our departments analyze approximately 2000 pediatric infection test requests annually, of which fifty eight (58) were selected depending upon history and clinical picture.

Study Population. The study attempted to enroll all eligible children between the ages of 1 day and 3 years who were admitted to pediatric department and were authorized by pediatrician to require venipuncture and lumbar puncture as part of their diagnostic work-up due to the presence of a fever or a history or clinical appearance. As per protocol described earlier (Lau, 2008; Lau *et al.,* 2008) fever was defined as either a confirmed rectal temperature ≥38.0ºC in the pediatric department or a history of a rectal temperature ≥38.0ºC. Exclusion criteria for study subjects was also taken as recommended earlier (See *et al.,* 2001) through set of following conditions: 1) no requirement for lumbar puncture; 2) no requirement for blood tests; 3) inability to obtain blood sample; 4) inability to obtain CSF sample; 5) history of recent seizure; 6) history of recent head trauma; 7) history of neurodegenerative disease; and 8) history of intraventricular hemorrhage. Eligibility criteria which were described earlier for infants with bacterial meningitis were as follows: clinical (respiratory distress, lethargy, presence/absence of minor/major neurologic symptoms, feeding and abdominal distension problems, temperature instability, unexplained recurrent hypoglycemia, poor vascular perfusion) and laboratory signs of septicemia with altered CSF results (leukocyte count, protein, glucose, visible bacteria) (See *et al.,* 2001).

Study groups. Infants were distributed into two groups according to presence or absence of meningitis. They were further divided into gram +ve, gram –ve and viral meningitis groups. Infants (around 10) without laboratory detection of infection in their CSF were used as the control group. Laboratory staff performing the analytical procedures for NSE were informed about the patient's final diagnosis based on CSF laboratory studies to avoid result bias. .

Sample collection. CSF and blood samples were collected from all selected patients as part of their routine evaluation for bacterial sepsis. Lumbar puncture was performed as per usual protocol at pediatric department, and the subsequent CSF was analyzed for cell count, protein, and glucose concentrations, NSE and cultures for bacteria and viruses. Clinical data were retrieved and documented.

Analytical Measurements: CSF NSE concentration was analyzed by an Electro-chemiluminescence technology (ECL) on Elecsys 2010 (Roche Diagnostic, Basil) according to the manufacturer's instructions. Samples were analyzed in duplicate and compared with known standards of NSE. The lower limit of detection of the ECL was 1.00 ng/mL for NSE. Glucose and protein concentrations were also estimated by using standard protocols on chemistry automated analyzer Hitachi 912 (Roche Diagnostic, Basil).

Data Analysis: Data are expressed as mean ± standard deviation. Statistical analyses were performed with SPSS ver 13.0 (USA). Difference between the groups was evaluated using student's "t" test and Pearson's correlation and statistical significance level was atleast $P< 0.05$.

RESULTS

The results are summarized in Table 1. In the present study causative bacteria and the number of children/infants effected by them were: gram-positive cocci, *Streptococcus pyogens* and *Streptococcus pneumonia*, in 6 and 13 cases respectively, gram-negative bacilli, *Haemophilus influenzae* and *Escherichia coli* in 4 and 5 cases, respectively; gram negative cocci *Nesseria meningitides* in 10 cases and viral meningitis in 20 cases. The control group consisted of infants in which, CSF samples had been collected to investigate meningitis, however their CSF leukocyte counts, protein and glucose concentrations were found to be normal and CSF culture or bacterial antigen test results came out to be negative (See *et al.,* 2001). An additional criterion, as per recommended in See *et al.,* (2001), was also used for admission to the control group was that the ultrasound patterns must be negative for encephalitis and central nervous system diseases.

In bacterial meningitis groups, the average hospital stay was ranged from 10.27 ± 5.0 to 15.20 ± 4.26 days, whereas it was less in viral meningitis group i.e. 8.26 ± 1.20 days. The control group which was devoid of any meningeal infections stayed at an average of 3.6 ± 1.10 days (Table 1). CSF protein and glucose components exhibited typical pattern of elevation when compared amongst the bacterial and viral meningitis groups, as well as with control group. Highly significant to moderately significant elevation in protein concentration was noted in patients suffering from *N. meningitides* (protein = 1012.10 ± 120.50 mg/dl) when compared with patients of gram –ve bacilli (P <0.001; ranges 560.50 ± 78.10 to 620.20 ± 49.21 mg/dl) and gram +ve cocci ($P< 0.01$; ranges 892.35 ± 56.50 to 961.80 ± 98.75 mg/dl) groups. Mildly significant elevation ($P< 0.05$) in protein concentration was also noted between meningitis by gram –ve bacilli (*E.coli*, 560.50 ±78.10 mg/dl) and gram +ve cocci (*S. pneumonia*, 961.80 ± 98.75 mg/dl). Furthermore, viral meningitis group also showed typical pattern of moderate to marked (P<

0.05 to P< 0.01) elevation in protein concentration (200.35 ± 38.60 mg/dl) as compared to bacterial groups except gram –ve cocci. However, the cumulative elevated protein concentration of bacterial group, when compared with viral group was noted to be extremely significant (*P*< 0.0001). Glucose concentration in CSF exhibited moderate significance when levels were compared among bacterial groups and viral meningitis. In bacterial meningitis groups of all etiology, predominant cells were polymorphonucleocytes (PMNs) whereas in viral group, lymphocytes. Cells count in all bacterial meningitis groups exhibited highly significant (*P*< 0.001) infiltration when compared with viral group. Mild significant difference (*P*< 0.05) was also noted in cell count of same gram –ve bacterial groups; one being gram –ve cocci, *N. meningitis* (5266.59 ± 230.10 cells/mm^3) and others two gram –ve bacilli, *E coli* (3439.56 ± 145.30 cells/mm^3) and *H. influenzae* (3620.29 ± 13.57 cells/mm^3).

Table 1. Etiology, biochemical contents, cells and NSE levels in CSF of patients with bacterial and viral meningitis

Etiology	No of patients	Hospital stay(days)	CSF changes			
			Protein Mg/dl	Glucose mg/dl	Cells cell/mm^3	NSE ng/ml
I-Gram +ve cocci						
a)Sterptococcus pyogens	6	10.27 ± 5.0	892.20 ± 56.50^{b}	17.21 ± 5.10	4096.10 ± 210.26^{a}	23.75 ± 6.20^{a,c}
b) Sterptococcus pneumonia	13	15.12 ± 2.80	961.90 ± 98.60^{b,c}	22.10 ± 3.60	4319.70 ± 189.60^{a,b}	30.40 ± 7.45^{a,c}
II-Grame-ve bacilli						
a) Hemophilis influenzae	4	13.21 ± 3.89	620.20 ± 49.21^{a,c}	18.29 ± 3.10	3620.29 ± 130.57^{a,c}	48.22 ± 5.90^{a}
b) Escerchia coli	5	12.11 ± 1.90	560.50 ± 78.10^{a}	25.26 ± 2.29	3439.56 ± 145.30^{a,c}	35.80 ± 6.10^{a,b}
III-Gram –ve cocci						
Nesseria meningitides	10	15.20 ± 4.26	1012.10 ± 120.20^{a,b}	10.19 ± 2.60	5266.59 ± 230.10^{a,b,c}	50.50 ± 8.96^{a,b}
IV-Viral meningitis	20	8.26 ± 1.20	200.35 ± 38.60^{a}	20.45 ± 1.20	101.26 ± 20.16^{a}	20.19 ± 2.22^{a,b}
V-control group	10	3.60 ± 1.10	30.30 ± 2.15	49.20 ± 4.60	5.20 ± 1.10	1.69 ± 0.91^{a}

Difference between the groups was evaluated using student's *t*-test. ^{a}Significantly differ at P<0.001 ^{b}Significantly differ at P<0.01 ^{c}Significantly differ at P<0.05. Normal references ranges; Protein 50-80 mg/dl; Glucose 10-40 mg/dl; Cells <5.0 cell/mm^3; NSE 1.0 ng/ml

CSF NSE concentrations showed marked elevation (*P*<0.001) in all meningitis groups irrespective of either viral or bacterial origin when compared with control group of non-infected patients. Moreover, mild (*P*< 0.05) to moderate (*P*< 0.01) significance level of elevation was also observed even within the meningitis group of viral (20.19 ± 2.02 ng/ml) versus *N. meningitis* group (50.50 ± 8.96 ng/ml) ; viral versus *E.coli* (35.80 ±6.10 ng/ml) and viral versus *S. pyogens* group (23.75 ± 6.20 ng/ml). The result of NSE being significantly elevated in meningitis patients from all etiological groups clearly support and advocate our hypothesis that meningitis infections and infiltration cause neuronal and neuro-physiological alterations and damages thus causing release and increased levels of NSE.

DISCUSSION

The present findings clearly suggest that NSE can be used as a potential marker in CSF as a possible diagnostic tool for the early detection of infants at risk of brain lesions from bacterial and viral meningitis. Bacterial meningitis being itself representing a major cause of perinatal mortality and morbidity, and causative factor for approximately one-third of affected infants develop neurologic sequelae (Escobar *et al.,* 2000)*;* needed to be evaluated and diagnosed with the help of additional and newer factors and tools. Besides being expressed selectively in neurons, NSE has a high stability in biological fluids and, as a free soluble cytoplasmic protein, can easily diffuse to the extracellular medium and cerebrospinal fluid (CSF) when neuronal membranes are injured. Hence, measurements of

CSF-NSE (cNSE) may be an attractive marker of neuronal damage (Marangos *et al.,* 1979; Marangos and Schechel, 1987). There are some peculiarities, however, that have to be considered when cNSE or other CSF neuronal markers are assayed: nature, location and extension of the lesion; CSF turnover and time elapsed between neuronal injury and CSF sample collection (Hardemark *et al.,* 1989; Royds *et al.,* 1981). It has been suggested that there may be an increase of cNSE at earlier stages of neuro- degenerative disorders, followed by a gradual decrease as the disease progresses.

Similar to our study, earlier investigation showed progressive outcome when NSE was estimated in CSF. The investigations were performed in 16 subjects suffering from bacterial meningitis. In all individuals CSF and plasma NSE concentration was estimated during the first 24 hours of hospitalization. Mean CSF NSE concentration in patients in very severe clinical state (group I) was 19.8 □g/L compared to 10.46 □g/L in subjects of group II with moderate and mild course of disease. The obtained results indicate the usefulness of CSF NSE concentration assessment in estimation of severity of the patient's clinical state especially in purulent, bacterial meningoencephalitis (Kepa, 2009).

Furthermore, the relationship between CSF-NSE levels and neurological complications or outcome was examined in childhood bacterial meningitis. It was noted that CSF-NSE levels were significantly higher in the patients with bacterial meningitis than in the patients with the other central nervous system (CNS) infectious diseases, suggesting that CNS damage in those patients with bacterial meningitis was exacerbated. As CSF-NSE levels increased to above 25 ng/mL in the acute phase, all patients except one had subdural effusion. In those patients whose CSF-NSE level rose again during the illness, CNS complications or sequelae occurred clearly suggesting that CSF-NSE may be a useful prognostic factor for predicting CNS damage in childhood bacterial meningitis (Inoue *et al.,* 1994).

It has been argued that NSE level not only suggests neuronal damage or infections but may also dependent on gestational age or birth. In this regard, activities of neuron-specific enolase (NSE) in the cerebrospinal fluid (CSF) were measured in 104 neonates (24 cases with CNS diseases and 80 cases without CNS diseases) aged from 0 to 27 days and 50 children without CNS diseases aged 3 months to 15 years. It was found that the mean level of NSE activity in the CSF of neonates with CNS diseases was significantly higher than that of neonates without CNS diseases. However, high levels activities gradually decreased with the clinical course (Inoue, 1992).

Similar to our studies to establish newer markers of neuronal infections in infants and children, in far and near past, several researcher and scientists considered another marker S100□□ equally significant to NSE, in assessing neural damage and CNS infections. In this regard, a recent past study demonstrated that children with septic shock showed increased serum S100β and NSE as compared with controls. To finalize the verdict of CNS marker efficacy, supported by the fact that both NSE and S100B markers of neurologic injuries are increased in children with septic shock, the authors argued that it may be indicative of sub-clinical injuries that are either transient or permanent. However it was also suggested that more extensive studies are needed, and in our view with larger groups, that could correlate the long-term neurologic outcome of children through detection of CNS markers. That would certainly facilitate the identification of children at risk for neurologic injuries from septic shock (Hsu *et al*., 2008).

To further assess the usefulness of NSE and S100β, as markers of CNS injury and its severity, its evaluation has also been extended to adult groups, where serum S100B and NSE concentrations had shown to be increased after Traumatic Brain Injury (TBI) (Ingebrigtsen and Romner, 1996; Persson *et al.,* 1987; Raabe *et al.,* 1998, 1999; Skogseid *et al.,* 1992). In these patients, there is a highly significant, direct correlation between the amount of these proteins in the serum and patient outcome (Raabe *et al.,* 1999). A significant study published in 2000 (Romner *et al.,* 2000) considering almost 800 adults with mild, moderate, and severe TBI found that increased serum S100β was an excellent predictor of computed tomography scan abnormalities, raising the possibility that S100β could also be used as a screening test for diagnosis of intracranial injury or CNS alterations per se (Romner *et al*., 2000). In the same year, a comparative usefulness study is not far away and pilot study in children shows an increase in serum NSE after TBI (Fridriksson *et al*., 2000), in addition to preliminary study that showed an increase in both serum and fluid NSE (Berger *et al.,* 2002). The study also demonstrated that NSE and S100β concentrations are markedly increased in the CSF of children after TBI. The report also suggested that both NSE and S100β may have the potential to be used as quantitative measures of the success of therapy for TBI (Berger *et al.,* 2002; Fridriksson *et al*., 2000; Romner *et al.,* 2000).

Conclusion

In conclusion bacterial meningitis represents a major cause of perinatal mortality and morbidity and responsible for approximately one-third of affected infants, that finally develops neurologic sequelae. Such diseases and conditions needed newer and faster tools to investigate the extent of damage or prognosis. The present study therefore investigated the possible diagnostic significance of cerebrospinal fluid's NSE and noted NSE being

significantly elevated in meningitis patients from all etiological groups. This supported the hypothesis that meningitis infections and infiltration cause neuronal and neuro-physiological alterations and damages thus causing increase in NSE concentrations. It is therefore suggested that NSE can be used as a potential marker in CSF as a possible diagnostic tool for the early detection of infants at risk of brain lesions from bacterial and viral meningitis.

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