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# Prognostic Value of Neuron-Specific Enolase (NSE) Biomarker in Neonates with Encephalopathy: Review Article

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## ABSTRACT

**Background:** Any illness that disrupts the central nervous system in the first few days of life can induce encephalopathy among neonates. This ailment can have a variety of causes. Of all the CSF neurobiochemical markers in babies with HIE, NSE has received the greatest amount of research. Up to four percent of all soluble brain proteins are NSE, a dimeric glycolytic enzyme. Neurons and neuroendocrine cells contain it primarily in their cytoplasm. When neurons suffer damage to their axons, NSE is found in high concentrations in their cytoplasm. Thus, it is sensitive for axonal injury and neuronal cell death and has a fairly high specificity for detecting axonal damage. Traditional terminology for neonatal encephalopathy (NE) has focused on hypoxic-ischemic encephalopathy, which occurs when an intrapartum incident causes perinatal hypoxia-ischemia.

**Objective:** The purpose of this study was to review the diagnostic value of the Neuron-Specific Enolase biomarker in the evaluation of early detection and its role in prognosis of neonatal encephalopathy.

**Methods:** In our search for information on NSE and its level in neonates with encephalopathy, we used Google Scholar, Science Direct, PubMed, and other internet databases. Additionally, the writers combed through relevant literature for references, however they only included researches that were either very recent or covering the years from 2010 to 2023.

**Conclusion:** Serum NSE is considered to be a reliable marker for the diagnosis of different stages of NE.

**Keywords:** Neonates, Encephalopathy, Neuron-Specific Enolase.

## INTRODUCTION AND NEONATAL ENCEPHALOPATHY:

It is now customary to refer to the malfunction of the central nervous system in infants as "neonatal encephalopathy". This syndrome, which is diverse and clinically characterized, is seen in newborns born at or after 35 weeks of gestation. It appears in the early stages of infancy with symptoms such as seizures or decreased consciousness, frequently accompanied by respiratory difficulties, a weakening of the muscles and reflexes, and decreased muscular tone [(1)].

This description is appropriate given our poor understanding of brain damage leading to neurologic disability in neonates, as it does not imply a specific underlying cause. Although hypoxia-ischemia was formerly thought to be the primary cause of newborn encephalopathy, it is now known to be merely one of many potential causes. It can be difficult to determine whether a specific case of newborn encephalopathy is caused by hypoxic-ischemic brain damage [(2)].

While, some studies consider a low five-minute Apgar score adequate, others impose specific criteria, such as requiring two or more symptoms lasting over 24 hours, for the diagnosis of newborn encephalopathy. But depending just on Apgar scores can be problematic because they can be normal even in the case of severe hypoxia-ischemic damage, or they can be low due to maternal analgesia or preterm [(3)].

When hypoxic-ischemic brain injury is proven to be the cause of newborn encephalopathy, the term hypoxic-ischemic encephalopathy (HIE) is appropriate [(4)].

Given the lack of certainty surrounding the exact cause and timing of neonatal encephalopathy, some medical professionals advise using terms like as "presumed HIE" or "apparent HIE" when describing the patient's symptoms and any relevant MRI. When the cause is unclear, some claim that the more encompassing name "neonatal encephalopathy" is more appropriate. Neuroimaging and other tests may not be able to determine if hypoxia during pregnancy, asphyxia at birth, or hypoxic-ischemic brain damage is the underlying cause. A condition of impaired blood gas exchange leading, if it persists, to progressive hypoxaemia and hypercapnia" is the definition of asphyxia. A blood gas test is necessary for diagnosis [(5)].

Nevertheless, there is currently no accurate way to detect cerebral blood flow, brain oxygenation, or brain function during the prenatal or intrapartum stages, not even with sophisticated monitoring. Consequently, phrases like "foetal distress" and "birth asphyxia" are not always used appropriately [(6)].

Studies employing brain MRI, near-infrared spectroscopy, and electroencephalogram monitoring reveal that the early neonatal phase is frequently crucial for the emergence of brain damage [(7)].

Clinical indicators of an early antenatal onset of neonatal encephalopathy include contractures, small head size (which may indicate injuries during the first trimester



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if both the head and body are tiny), intrauterine growth restriction, and arthrogryposis-like characteristics [(2)].

**ETIOLOGY:**

Several factors have the potential to cause neonatal encephalopathy. While, other conditions can cause neonatal encephalopathy, hypoxic-ischemic encephalopathy (HIE), sometimes called birth asphyxia, is the leading cause. Because it does not suggest a particular underlying cause or pathophysiology, the term "neonatal encephalopathy" has become the go-to for describing the clinical syndrome of central nervous system dysfunction in the newborn period. This is helpful because the exact nature of brain injuries that cause neurologic impairment in newborns is often not well understood [(8)].

**HYPOXIC-ISCHEMIC ENCEPHALOPATHY:**

Acute hypoxia-ischemia is the most researched cause of neonatal encephalopathy, while there are other possible causes as well. Many people have used the term "neonatal encephalopathy" interchangeably with "hypoxic-ischemic encephalopathy" over the years. The reasoning behind this is that various etiologies are often documented as a distinct diagnosis when dealing with metabolic abnormalities (such as non-ketotic hyperglycemia) or infections (such as meningitis) among other specific causes. Accordingly, it is essential to emphasize that newborn encephalopathy can result from a wide variety of processes that might arise before, during, or immediately after birth. These processes are mostly, but not entirely, caused by genetic, metabolic, viral, and traumatic processes. The lack of homeostasis is a common feature of newborn encephalopathy, and it may result in aberrant brain function and even anatomical alterations in the brain [(9)].

**GENETICS:**

A genetic mutation in the IL-6 coding gene was associated with abnormal findings on transfontanellar ultrasonography and electroencephalography in term neonates diagnosed with NE clinically. Carriers of the IL6 174G > C mutation (CC genotype) also had a higher risk of neurological symptoms compared to those with the IL-6 174 GC genotype. Mutations in the MECP2 gene, which is associated with X-linked Rett syndrome, can cause severe NE in men. Early death, microcephaly, seizures, hypotonia, and motor deficits are symptoms experienced by infants afflicted [(10)].

**INFLAMMATION:**

Although there are a variety of possible causes of NE, the systemic inflammatory response in infants appears to be proportional to the severity of the condition. Furthermore, in newborns with NE, cytokine responses predict seizures, MRI abnormalities, and neurodevelopmental outcomes [(11)]. Neutropenia in newborns after NE is linked to poor neurological outcomes. On the other hand, inflammation has long been understood to be essential for both its dual function in repair and as a catalyst for neuronal damage in prenatal brain injury [(11, 12)].

**EPIGENETICS:**

Epigenetic modifications are changes to the genome that are both heritable and functionally relevant, they do not involve changes to DNA sequence. Epigenetic modifications, such as DNA methylation and demethylation, histone modifications, and microRNAs, establish persistent patterns of gene expression that enable cellular development [(13)]. Environmental factors can affect epigenetics: Stressful situations including low oxygen levels, infections, and bleeding can alter a fetus's DNA throughout its development and raise the possibility of neurological problems later in life, such as neonatal epilepsy [(14)].

**TRANSCRIPTOMICS:**

By utilizing whole blood RNA from 12 babies with NE and 12 healthy controls, 950 genes were identified through next-generation sequencing as having significantly different expression levels between the two groups. The expression of several key regulators of the hypoxia response varied significantly. Among these genes, 29% were found to be overexpressed in NE, while 71% were under expressed [(15)].

**METABOLIC DISORDER:**

In NE, the first reaction to hypoxia damage is metabolic alteration. Glucose and oxygen supply disruptions set off a cascade of harmful metabolic reactions. Anaerobic metabolism must take over when oxygen is scarce because it interferes with the tricarboxylic acid cycle and electron transport chain's regular operation. Rapid ATP depletion results in the failure of the ion pumps. When ionic transport is blocked, a cell's membrane depolarizes, causing an increase in $Ca^{2+}$, extracellular glutamate, and free radicals. Any one of these steps could lead to cell death. Metabolic pathway analysis of cord blood revealed 29 putatively annotated metabolic features that were significantly different in the perinatal asphyxia group compared to healthy controls. Eight of these characteristics were similarly significant in the HIE group. By comparing the HIE group to healthy controls, we can see that 50% of the tryptophan pathway metabolites and 75% of the pyrimidine pathway metabolites have changed [(16)].

## EVALUATION OF THE NEONATE WITH ENCEPHALOPATHY:

Given the variety of encephalopathy aetiologies, a comprehensive evaluation is required, especially in cases where prenatal asphyxia is unlikely or when there hasn't been a sentinel episode [17].

## EXAMINATION AND TAKING A HISTORY:

Neonates should have a thorough neurologic examination as well as a meticulous assessment for indications of aberrant fetal development, such as birthmarks, dysmorphic facial features, and congenital abnormalities of the skeleton and internal organs. Persistence of reduction in fetal movements may be indicated by a palmar crease absence, joint contractures, and micrognathia, which may indicate the prenatal beginning of encephalopathy [18].

## ASSESSMENT OF A LABORATORY:

Vital information on the fetus's perfusion method can be obtained from both the base and the umbilical artery pH. Blood urea nitrogen (BUN), liver enzymes, creatinine, bilirubin levels, coagulation profile, glucose, and electrolyte panel tests are among the many laboratory evaluations that are performed in addition to newborn lactate levels and blood gas, a full CBC, calcitonin, blood cultures, and C-reactive protein to check for indicators of infection. The purpose of these tests is to look for indications of infection. Lumbar punctures are necessary for the performing of cell counts, cultures, and viral testing for a variety of viruses, including herpes simplex virus, rotavirus, parovirus, and others, in cases when a possible infection of the central nervous system is suspected [19].

## NEUROIMAGING:

It is advised that all infants experiencing seizures or encephalopathy have MRI to better understand the cause of the condition and its prognosis. While, head ultrasonography can be helpful in detecting hemorrhage or ventriculomegaly on the spot, it is frequently normal in the immediate aftermath of a hypoxic-ischemic event [18]. Computed tomography (CT) is infrequently used on infants because large radiation doses are necessary to get acceptable resolution of the brain parenchyma [20].

Seizures or early encephalopathy may result from structural-developmental problems seen on traditional T1 and T2 scans, in addition to increasing the risk of further hypoxic-ischemic injury at delivery. Acute phase, spanning the initial seven to ten days following an accident, is when DWI is most useful for localizing specific injury sites. Reduced neuronal integrity is shown by low N-acetyl aspartate (NAA), and aberrant metabolism, most commonly caused by acute damage, is indicated by a lactate peak, both of which can be detected by magnetic resonance spectroscopy [21].

Depending on the severity and pattern of injury, MRI can forecast future deficits, making it a valuable prognostic tool. Neonates that undergo therapeutic hypothermia show less damage on MRI, especially in the white matter and watershed regions, as well as in the thalamus and basal ganglia [20].

## HYPOXIC ISCHEMIC ENCEPHALOPATHY:

Neonatal encephalopathy is a disorder that affects the central nervous system in the first few days of life and can be caused by a number of disorders. Hypotonia, seizures, abnormal primitive reflexes, feeding problems, apnea, and unusual weeping are the characteristic signs of newborn encephalopathy. Alterations in mental status, such as agitation, coma, and reduced responsiveness, are also present. Newborn encephalopathy, according to **Glass** [22], can be either temporary and reversible or the precursor to brain damage or malfunction that results in permanent disability.

## PATHOPHYSIOLOGY:

In order for the foetal brain to metabolise lactate, ketone bodies, and glucose, it requires a constant supply of ATP. The foetal brain is more resilient to hypoxia-ischemia (HI) than the adult brain because it has the capacity to store energy for usage when necessary. However, a significant ATP deficit can also cause damage to the developing foetal brain [23]. The pathophysiology of HIE is primarily composed of five processes: Oxidative stress, excitotoxicity, intracellular $Ca^{2+}$ accumulation, mitochondrial malfunction, and inflammation. These occurrences are all related to one another [24].

## MECHANISMS OF NEURONAL INJURY:

The hypoxia-ischemia response first causes energy failure and mitochondrial dysfunction. This is linked to a number of harmful effects, including membrane depolarization, brain oedema, increased release and inhibition of neurotransmitters, and an increase in intracellular calcium that starts other cascades of pathology. Some examples of this are oxidative stress and the reactive oxygen and nitrogen species it generates, as well as the nitric oxide pathway and its interactions with reactive nitrogen species [25].

The successive harmful cascades and the ensuing clinical symptoms are determined by the fetus's response to the hypoxic-ischemic insult. A study used apparent diffusion coefficient (ADC) mapping as a measure of ischaemic injury in conjunction with MRI diffusion-weighted sequences to track the response of rabbit foetus brains to global hypoxia while they were still in the uterus. After 40 minutes of worldwide hypoxia, foetuses with a sharp decline in brain ADC displayed hypertonia and

postural abnormalities, while foetuses without such decline showed rather normal brain ADC at delivery. Thus, the initial energy failure and oxidative stress following the hypoxic-ischemic insult likely play a crucial role in subsequent cascades [25].

## NEURON-SPECIFIC ENOLASE AS A BIOMARKER:

Over the past few decades, the use of non-invasive laboratory biomarkers has grown to be crucial to clinical practice. One of the main goals of many current studies is to find novel biological markers that will allow for the early identification of newborns who are at risk of neonatal disorders, close monitoring of the condition, and prognostic information. One of the most important areas of modern scientific research is the development of early biochemical indicators of disease, as these markers may provide valuable and timely diagnostic information when clinical and radiological indications are still absent [26].

## NEURON-SPECIFIC ENOLASE IN NEONATAL ENCEPHALOPATHY:

Since NSE is a very specific marker for neurones [APUD (Amine Precursor Uptake & Decarboxylation) cells, and peripheral neuroendocrine tissue] it can be used as a biochemical marker for cancers arising from these cells. While, NSE like chromogranin A (CgA) is not a general neuroendocrine marker that can distinguish between various subtypes of NETs, it has been linked to inadequate tumor differentiation when levels of NSE are high. Immunostaining shows that all neuronal types—sensory, autonomic, granule, Purkinje, and projection neurons—contain NSE. Numerous normal cells have been discovered to display NSE including adrenal medullary chromaffin cells, pinealocytes, pituitary glandular and peptide-secreting cells, skin's Merkel's cells, lung neuroendocrine cells, and red blood cells [27].

Importantly, results from immunoassays and enzymatic techniques cannot be directly compared. The immunoassay measures the concentration of immunoreactive enolase independent of the protein's enzymatic activity. Nanograms of immunoreactive enzyme protein per milliliter is the standard unit of measurement for immunoassay results, in contrast to units of labor (U/L) for enzymatic testing. To quantify NSE in human plasma, a solid-phase immunobioluminescent assay was developed [28].

A sandwich-type enzyme immunoassay for NSE in human serum was created by utilizing purified antibodies to γ-enolase, an enzyme found exclusively in bovine neurons. After that, a new enzyme immunoassay called galactosidase was created to quickly measure NSE in serum with the help of monoclonal antibodies. **Torsetnes *et al.*** [29] suggested an innovative method for determining NSE. There were two parts to the process: The first was employing immobilized anti-γ-antibodies to capture the isoenzymes in the γ-subunit. The second part was testing the supernatant of samples treated with antibodies and untreated samples for enolase activity using a bioluminescence assay.

The specificity and sensitivity of this system's antibody for measuring NSE were enhanced by the fact that it only associated with the γ-subunit when combined with the avidin-biotin attached peroxidase complex. An immunoradiometric assay (IRMA) was performed using monodisperse magnetisable particles as the solid phase, and monoclonal antibodies against neuron-specific enolase were used. The assay sensitivity was 0.4 εg/L, and the intra-assay coefficient of variation (CV) was less than 5% within the working range of 0.4 to 170 μg/L. When comparing healthy individuals to those with small cell lung cancer (SCLC), patients using an IRMA that was adapted from Paus and Nustad's method demonstrated a 93% sensitivity increase compared to the RIA's 83%. In 2012, a new chemiluminescence enzyme immunoassay was developed using magnetic nanoparticles to detect NSE in human serum: An NSE detection antibody is labeled with alkaline phosphatase and an NSE capture antibody is coupled to the NSE using fluorescein isothiocyanate (FITC) in a sandwich-type detection approach. Adding anti-FITC-coated magnetic beads to this immunological complex and then enriching it in a magnetic field increases the sensitivity. The recovery rate, limit of detection, and CV for this approach were all over 83.0%, with the latter two being below 0.2 ng/mL and 10%, respectively. The high selectivity of this immunoassay is unaffected by the hook effect [29].

As a result, NSE concentrations shouldn't be measured on samples that are obviously hemolyzed or that are stored at room temperature. They discovered that following a month of storage at -20 °C, the concentration of NSE in CSF dramatically decreased (−27.6%). Serum's NSE concentration remained at -80°C for a minimum of nine months. These figures demonstrate how erratic NSE is in CSF. The sample's protein concentration and aliquot volume may have an effect on NSE stability. In any event, the temperature and length of the freezing period affect the NSE level that is measured. Additionally, the authors noted a substantial rise in NSE concentration in both serum and CSF samples that had been hemolyzed as well as a strong association between the increase in NSE concentration and the hemolysis index. They found that, for NSE measurement, all samples should be kept frozen for up to nine months; in the case of CSF, samples should be evaluated within six months of sampling and stored at −80 °C. Moreover, before choosing to do an NSE measurement in serum or CSF, it is imperative to thoroughly assess the index of hemolysis [29]. More recently, **Planche and colleagues** [30] verified that the serum NSE levels increased in tandem with the hemolytic

index (about 150 % for a hemolytic index of 10), the conclusion that, in order to enable accurate tracking of the marker's kinetics, NSE calculations should only be carried out for samples with a hemolytic index of ≤10. There was no gender-specific intra-individual variance or difference in blood NSE concentrations between men and women [(31)].

## CLINICAL INDICATIONS FOR NSE DETERMINATION:

When assessing a tumor's neuroendocrine differentiation, NSE—a pan-neuroendocrine tissue marker—is essential. Another way of looking at it is as a general indicator of secretion. The pattern of secretion varies depending on the disease's stage and its embryological genesis. In SC neuroendocrine carcinomas characterized by large cells and inadequate differentiation, NSE has been found to be a more sensitive differentiation marker than CgA [(32)].

## BRAIN DAMAGE:

Glia, neurones, and myelin release NSE, tau-protein, myelin-basic protein, glial fibrillary acidic protein, and S100-β into the bloodstream following an ischemic stroke. These are some potential signs of damage to the brain tissue. It is possible to learn about the severity of brain infarction from the blood concentration of these markers. Finding blood markers to measure treatment efficacy in the early phases of acute stroke would also be a simpler and cheaper alternative to other surrogate endpoints such radiographic evaluations of tissue damage [(32)]. **Ahmad *et al.*** [(33)] showed that in the first week after a stroke, there is a correlation between the infarct volume radiological assessments and the peak levels of NSE and S100-β levels. Plasma biomarker measurements taken in the initial six hours following a stroke are unlikely to be accurate predictors of the severity of a subacute infarct, according to their findings. However, NSE has proven to offer quantitative assessments of brain damage and/or enhance diagnosis and outcome evaluation in various clinical contexts, including those involving intracerebral hemorrhage, seizures, comatose patients following cardiopulmonary resuscitation for cardiac arrest, and traumatic brain injury [(34)].

## CONCLUSION

Serum NSE is considered to be a reliable marker for the diagnosis of NE.


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