



# “Evaluation of Gfap Levels in Predicting Prognosis in Perinatal Asphyxia - Hypoxic Ischemic Encephalopathy”

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**Keywords:** GFAP (Glial Fibrillary Acidic protein); Neurodevelopment; Hypoxic encephalopathy; Perinatal Asphyxia; Perinatal hypoxic event.

## Abstract

**Aim:** To study the possible role of GFAP in predicting neuro developmental outcome in perinatal asphyxia complicated by hypoxic encephalopathy.

**Objectives:** To assess:

1. To study the levels of GFAP in neonates affected with perinatal asphyxia.
2. To guide the optimal timing in relation to detection of GFAP after an acute perinatal hypoxic event.

**Methods:** The study was a prospective case control study conducted in the Department of Pediatrics, Sheri Kashmir Institute of Medical Sciences, Soura, Srinagar. The study was undertaken for the newborns admitted in NICU, Department of Pediatrics with features of perinatal asphyxia fulfilling the criteria defined by the American Academy of Pediatrics (AAP) & American College of Obstetrics and Gynecology (ACOG). The control group in the study consisted of neonates admitted in NICU without any features of neurophysiological affliction. Venous Blood samples of approximately 2 ml were collected on two occasions at 12 hours and 48 hours of life in red tubes (containing clot activator), allowed to clot, centrifuged and stored in a monitored facility at -80°C until analysis for Glial Fibrillary Acidic Protein (GFAP). The GFAP levels were compared in the cases at 12 and 48 hours of life and similarly in controls. The relative levels were then compared between cases and controls at same time points i.e. 12 and 48 hours of life. The cases were periodically followed up after discharge and evaluated at 3 & 6 months for neurodevelopment outcome by using the Hammersmith Infant Neurodevelopmental Scale (HINE).



**Result:** The mean serum GFAP level in the HIE group was 0.253 ng/mL while the mean GFAP level in the control group was 0.056 ng/ml at 12 hours of life. The difference between the two groups was statistically significant. ( $p < 0.001$ ). The mean serum GFAP level in the HIE group was 0.285 ng/mL while the mean GFAP level in the control group was 0.050 ng/mL at 48 hours of life. The difference between the two groups was statistically significant ( $p < 0.001$ ).

**Conclusions:** The mean serum GFAP level in the HIE group was statistically significant at 12 as well as 48 hours of life. Increased GFAP levels in postnatal period at both 12 and 48 hours of life were negatively correlated to neurodevelopmental outcome at 3 and 6 months of life.

## Introduction

Asphyxia is a Greek word meaning “without pulse” [1]. WHO has defined perinatal asphyxia as a “failure to initiate and sustain breathing at birth”. As per the American Academy of Paediatrics (AAP) & American College of Obstetrics and Gynaecology (ACOG), the following must be present for designation of Asphyxia:

- A) Profound metabolic or mixed acidaemia ( $p^H < 7.00$ ) in cord blood.
- B) Persistence of APGAR scores 0-3 for longer than 5 minutes.
- C) Neonatal neurological sequelae (e.g., seizures, coma, hypotonia)
- D) Multiple organ involvement (e.g., of the kidney, lungs, liver, heart, intestine) [2].

An executive summary that describes the newborn symptoms and contributing factors used to determine acute hypoxia-ischemic episodes in term and late preterm infants (GA > 35 weeks) that would likely result in Hypoxic Ischemic Encephalopathy (HIE) was released by the ACOG Task Force on newborn Encephalopathy.

*Neonatal signs that point to an acute peripartum or intrapartum incident include:*

- An APGAR score of less than five at five and ten minutes.
- Foetal umbilical artery acidemia
- Base deficit  $\geq 12$  mmol/L, or  $p^H < 7.0$  in the foetal umbilical artery, or both.
- Acute brain injury detected by neuroimaging on an MRI or MRS that is consistent with hypoxia-ischemia.
- Multisystem organ failure with hypoxic ischemic encephalopathy present.

**Contributing factors that are consistent with an acute peripartum or intrapartum episode include the following types and timings:**

- A sentinel ischemia or hypoxic episode that happens just prior to or during childbirth:
- Uterine rupture.
- Severe placental abruption.
- Prolapse of the umbilical cord.

- Embolus of amniotic fluid concomitant with severe and protracted hypotension and hypoxemia in the mother.
- Cardiovascular collapse in mothers.
- Exsanguination of the foetus due to either significant haemorrhage from the mother or vasa previa.

Peripartum or intrapartum patterns on foetal heart rate monitors that are consistent with an acute incident, especially a category I pattern or presentation that changes into one of the following patterns:

- Pattern for Category III
- Tachycardia accompanied by frequent slowdowns
- A continuous minimal variability accompanied by periodic decelerations

Based on imaging investigations, the timing and kind of brain injury patterns are compatible with the aetiology of an acute peripartum or intrapartum event. Brain MRI findings that are well-defined and indicative of hypoxic-ischemic cerebral injury in neonates include: -Deep nuclear grey matter injury (basal ganglia or thalamus) Cortical injury in the border zone (watershed).

Severe birth asphyxia, as described by the WHO classification of disorders ICD 10, is characterised as an APGAR score of 0–3 at 1 minute. When the APGAR score at one minute is 4-7 [3], the birth hypoxia is mild to severe. Ten to sixty percent of infants with HIE pass away in the first few months of life [4] Asphyxia continues to be a serious illness that causes a great deal of death and morbidity, even with the tremendous advancements in prenatal treatment over the previous few decades. With a frequency of 1 to 6 per 1,000 live full-term births, perinatal asphyxia ranks third in terms of causes of neonatal death (23%) behind serious infections (26%), and preterm delivery (28%) [5].

Prediction of neurologic sequelae in full-term asphyxiated infants is traditionally based on clinical findings, neuro-physiologic examinations, and brain imaging. In severely ill infants, the first two of these parameters are affected by medications and therapeutic interventions, such as antiepileptic pharmacotherapy, ventilator treatment, and muscle paralysis. This has led to an intense search for a valid biochemical method that could give accurate information on prognoses [6].

Various biochemical markers that are detectable in blood and/or CSF have been investigated for improvement of neurological prognostics [7]. One such biomarker hypothesized to provide at least some degree of information about the process is the Glial fibrillary acidic protein. Glial Fibrillary Acidic Protein is the hallmark intermediate filament protein in astrocytes, a main type of glial cells in the central nervous system [8]. Astrocytes have a range of control and homeostatic functions in health and disease. GFAP is the main intermediary filament of astrocytes, the most abundant cell type in the human Central Nervous System (CNS). It plays an essential role in maintaining shape and motility of astrocytic processes and contributes to white matter architecture, myelination and blood-brain barrier integrity. Since relevant extra cerebral sources of this protein have not been uncovered, it was discovered that GFAP is very brain specific. Because of this, blood levels of GFAP in healthy people are often very low and do not raise over the lower detection limits of the tests that are utilised. It is believed that (I) astrocytic structural integrity loss from necrosis and/or me-

chanical disruption and (II) blood-brain barrier disintegration are necessary for the release of GFAP from brain tissue into the blood stream. GFAP release and the appearance of detectable protein levels in peripheral blood may result from the elevation of GFAP that occurs after various clinical events in the CNS, a process known as reactive astrogliosis. This possibility is currently unclear, though [9].

### Materials and methods

This was a prospective case control study conducted in NICU of a tertiary care hospital over a period of 2 years. A total of 38 patients with history of perinatal asphyxia were enrolled in study of which 3 patients were lost to follow up. Equal number of controls was enrolled in the study from NICU. Patients with evidence of sepsis, suspected or proven inborn errors of metabolism, brain malformations, neurological afflictions and other congenital anomalies were excluded from both case and control groups. The study was undertaken for the newborns admitted in NICU, Department of Paediatrics with features of perinatal asphyxia fulfilling the following criteria defined by the American Academy of Paediatrics (AAP) & American College of Obstetrics and Gynaecology (ACOG)

- 1) Profound metabolic or mixed acidemia ( $p^H < 7.00$ ) in cord blood.
- 2) Persistence of APGAR scores 0-3 for longer than 5 minutes.
- 3) Neonatal neurological sequelae (e.g. seizures, coma, hypotonia)
- 4) Multiple organ involvement (e.g. of the kidney, lungs, liver, heart, intestine).

The control group in the study consisted of neonates admitted in NICU without any features of neuro-physiological affliction or sepsis (negative sepsis screen and/or blood cultures) like TTN, neonatal jaundice etc.

The newborns that fulfilled the enrolment criteria for pre-natal asphyxia as defined by the ACOG criteria were included in our study as cases. A detailed history of the cases was taken including maternal age, birth order, gestational age, place of birth, birth spacing, parental consanguinity, previous maternal abortions or still births, high risk factors including Gestational Diabetes, Pregnancy Induced Hypertension, Preeclampsia etc., method of delivery and the method of anaesthesia used during labour if any. APGAR scores & the methods and duration of resuscitation were noted. The newborns underwent a complete physical examination including assessment of vitals, oxygen requirement, presence of any respiratory distress, perfusion, cardiac status, urine output, serial body weight etc on admission & at each day of NICU stay till discharge. Neurological status was assessed on admission and daily thereafter with the help of The Sarnat and Sarnat Staging and Thompson scoring. Sarnat and Sarnat staging assesses level of consciousness, neuromuscular control, reflexes, autonomic function, bronchial and salivary secretions, gastrointestinal motility and seizures and was used to classify the cases into HIE 1, 2 or 3 which is in the order of increasing severity. Thompson scoring assesses tone, level of consciousness, fits, posture, moro, grasp, suck, respiration and fontanels. Thompson score was used to classify the patients in mild, moderate and severe groups. Baseline investigations including hemogram, blood chemistry, kidney function tests, blood gases and electrolytes were recorded. Other investigations during hospital stay including coagulation profile, EEG, MRI etc.

were done on a case to case basis as per feasibility and where clinical condition demanded so.

Controls matched to cases in respect to sex, mean gestational age, birth weights, method of birth and inborn/out born status were taken. Patients with evidence of sepsis, suspected or proven inborn error of metabolism, brain malformations, neurological afflictions and other congenital anomalies were excluded from both case and control groups.

Venous Blood samples of approximately 2 mL were collected on two occasions at 12 hours and 48 hours of life in red tubes (containing clot activator), allowed to clot, centrifuged and stored in a monitored facility at  $-80^{\circ}\text{C}$  until analysis for Glial Fibrillary Acidic protein (GFAP).

The GFAP kit was based on Enzyme-Linked Immunosorbent Assay (ELISA). The plate was pre-coated with human GFAP antibody. GFAP present in the sample was added and bound to antibodies on the wells. And then biotinylated human GFAP antibody was added and bound to GFAP in the sample. Then Streptavidin in-HRP was added and bound to the Biotinylated GFAP antibody. After incubation, unbound Streptavidin in-HRP was washed away during a washing step. Substrate solution was then added, and colour developed in proportion to the amount of human GFAP. The reaction was terminated by addition of acidic step solution and absorbance measured at 450 nm. The GFAP values were expressed as ng/ml.

The GFAP levels were compared in the cases at 12 and 48 hours of life and similarly in controls. The relative levels were then compared between cases and controls at same time points i.e., 12 and 48 hours of life.

The cases were periodically followed up after discharge and evaluated at 3 & 6 months for neurodevelopmental outcome by using the Hammersmith Infant Neurodevelopmental Scale (HINE). The HINE scale consists of both scored and not scored components. The scoring system measured the infants objectively in the following 5 parameters:

- 1) Cranial Nerve Function ( Maximum Score 15) - Score of 0, 1, 2 or 3 given by assessing facial appearance, eye movements, visual movements, auditory response and sucking/swallowing.
- 2) Posture (Maximum Score 18) - Score of 0, 1, 2 or 3 by assessing head, trunk, arms, hands, legs and feet.
- 3) Assessment of Movements (Maximum Score 6) - Score of 0, 1, 2 or 3 given by observing the quantity and quality of movements.
- 4) Assessment of Tone (Maximum Score 24) - Score of 0, 1, 2 or 3 given by assessing Scarf sign, passive shoulder elevation, pronation/supination, hip adductors, popliteal angle, ankle dorsiflexion, pull to sit and ventral suspension.
- 5) Reflexes and Reactions (Maximum Score 15) - Score of 0, 1, 2 or 3 given by assessing arm protection, vertical suspension, lateral tilting, forward parachute and tendon reflexes.

The global score is measured as the sum of these 5 component scores to a maximum of 78.

Motor milestones and assessment of behavior are not scored. Motor milestones include the following: Head control, sitting, voluntary grasp, ability to kick in supine, rolling, crawling, standing and walking. Number of asymmetries assessed.

Behaviour includes assessment of conscious state, emotional state and social orientation.

A HINE score less than 67 at 3 months and less than 70 at 6 months was considered not optimal and consistent with neurodevelopmental delay and strongly associated with cerebral palsy based on review of previous studies regarding the application of HINE scoring [10].

The GFAP levels (at 12 & 48 hours) in cases were correlated to neurodevelopmental outcome as assessed by the HINE scoring (3 & 6 months of life).

Related investigations including MRI, EEG, BERA were recorded on follow-up contact whenever done and correlated to our findings.

**Results and observations**

We enrolled a total of 38 patients with history of perinatal asphyxia in our study of which 3 patients were lost to follow up. Equal no of controls were enrolled in the study from our NICU. Patients with evidence of sepsis, suspected or proven in-born errors of metabolism, brain malformations, neurological afflictions and other congenital anomalies were excluded from both case and control groups. Based on our primary objective we measured serial GFAP levels in CASES and CONTROLS on two occasions at 12 and 48 hours of life and compared them. We followed the patients for Neurodevelopmental delays based on the objective HINE (Hammersmith Infant Neurodevelopmental Scale) score. All standard statistical tests were run on our results and analysis was done using IBM SPSS version 21 and Microsoft Excel. A P value of less than 0.05 was considered significant. Underneath Tabulation and graphical representation presents the data recorded and analysis.

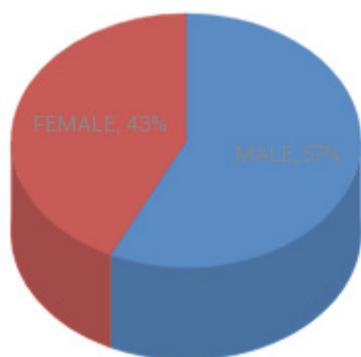
**Gender distribution**

Our study included 57 % males and 43% females in the case group while in the control group there were 51% males and 49% females. The difference was not statistically significant.

**Table 1**

Particulars	Case-n(%) (N=35)	Control-n(%) (N=35)	P value
Male	20(57)	18(51)	0.61
Female	15(43)	17(49)	

**Case group N=35**



**Figure 1**

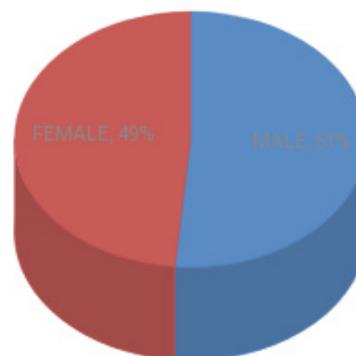
**Birth weight**

The mean birth weights in cases and controls are represented in the table and chart below. The difference is not statistically significant.

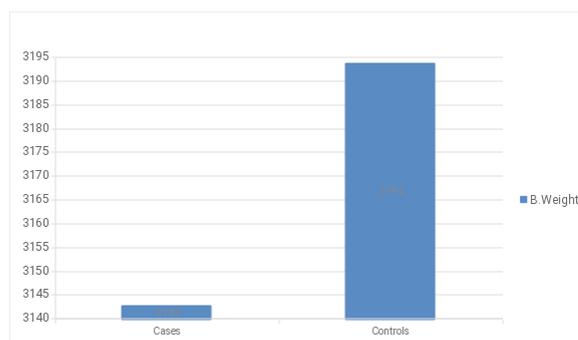
**Table 2**

Birth Weight (mean± SD)	Case group	Control group	P value
	3143 ± 199 gms	3194 ± 255gms	0.35

**Control group N=35**



**Figure 2**



**Figure 3:** Comparison of mean birth weights between the case and control groups.

**Method of delivery**

Underneath table and chart show the distribution of cases and controls in our study as per the method of delivery.

**Table 3**

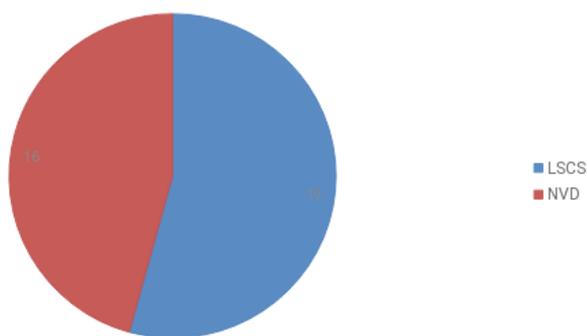
	Cases (n/%) N=35	Control (n/%) N=35	P value
LSCS	21(60)	19(54.2)	0.62
NVD	14(40)	16(45.8)	

**Cases**



**Figure 4:** Distribution of cases according to mode of delivery.

Controls



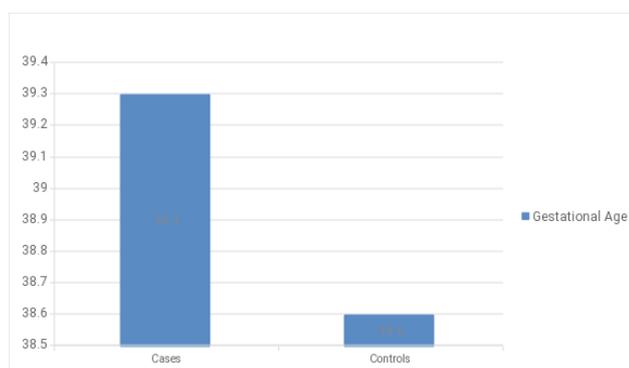
**Figure 5:** Distribution of controls according to mode of delivery.

**Gestational age**

The mean gestational ages in our cases and controls were comparable and the difference was not statistically significant.

**Table 4**

Birth Weight (mean± SD)	Cases	Controls	P value
Gestational Age (Mean±S.D)	39.3±1.4 weeks	38.6±1.1 weeks	0.71



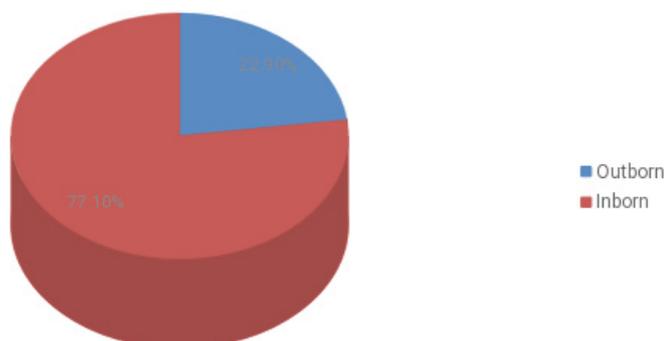
**Figure 6:** Comparison of mean gestational ages between cases and controls.

**Place of delivery**

Underneath table and chart shows the distribution of cases and controls as per the place of delivery.

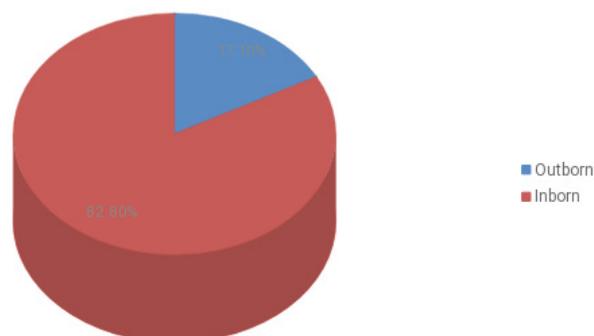
**Table 5**

	Cases (n/%)	Controls (n/%)	P value
Outborn	8 (22.9%)	6 (17.1%)	0.54
Inborn	27 (77.1%)	29 (82.8%)	



**Figure 7:** Distribution of cases according to the place of delivery.

Controls



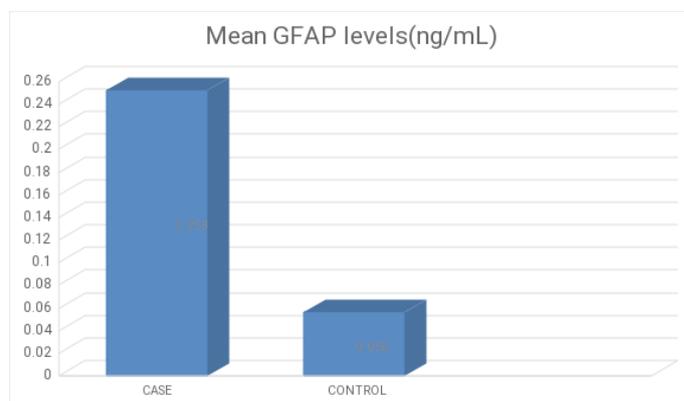
**Figure 8:** Distribution of controls according to the place of delivery.

**Mean GFAP levels in CASE and CONTROL patients at 12 hours of life**

**Table 6**

Group	No. of patients	Mean GFAP levels ± S.D(ng/mL)	P value
CASE	35	0.253±0.090	<0.001
CONTROL	35	0.056±0.015	

In this table and chart work Mean GFAP levels in case and control have been analysed. In control patients n=35; mean GFAP levels were 0.056 ng/mL, whereas in CASE patients n=35; mean GFAP levels were 0.253 ng/mL, which also depicts a significant increase in GFAP levels in CASE patients against the CONTROL ones. p.value was < 0.001



**Figure 9:** Comparison of mean GFAP levels in Cases and Controls at 12 hours of life.

**Mean GFAP Levels in CASE and CONTROL Patients At 48 Hours of Life**

**Table 7**

Group	No. Of patients	Mean GFAP±S.D(ng/mL)	P value
Cases	35	0.285±0.082	<0.001
Controls	35	0.050±0.011	

In this table and chart work Mean GFAP levels in case and control have been analysed. In control patients n=35; mean GFAP levels were 0.050 ng/mL, whereas in CASE patients n=35; mean GFAP levels were 0.285 ng/mL, which also depicts a significant increase in GFAP levels in CASE patients against the CONTROL ones. p.value was <0.001.

neurodevelopmental outcome had GFAP levels more than 0.24.

## Discussion

Even with the notable advancements in perinatal care over the past few decades, asphyxia is still a serious illness that causes a considerable amount of morbidity and mortality. The condition known as perinatal asphyxia is defined by a decrease in the exchange of oxygen and carbon dioxide in the respiratory system, leading to hypoxemia, hypercapnia, and metabolic acidosis [11]. According to research, asphyxia ranks third in frequency of causes of infant death (23%) after severe illnesses (26%) and preterm birth (28%) [12]. According to the World Health Organization, perinatal asphyxia accounts for over a million newborn deaths annually and is a major cause of long-term neurologic disability and impairment in children [13].

Perinatal asphyxia affects virtually every organ of the body including brain, kidneys, heart, respiratory and gastrointestinal system. A study showed that involvement of one or more organs occurred in 82% of the infants; the Central Nervous System (CNS) was most frequently involved (72%) Renal involvement occurred in 42%, pulmonary in 26%, cardiac in 29%, and gastrointestinal in 29% of the infants; 15% neonates had renal failure and 19% had respiratory failure [14]. The majority of organ involvement in perinatal asphyxia may be reversible, but CNS involvement may increase and result in a developing encephalopathy with long-term consequences that include hearing and vision loss, seizures, autism, ADHD, neurodevelopmental delay, and cerebral palsy. An estimated 1 million children who survive birth asphyxia live with chronic neuro-developmental morbidities, including cerebral palsy, mental retardation, and learning disabilities [15].

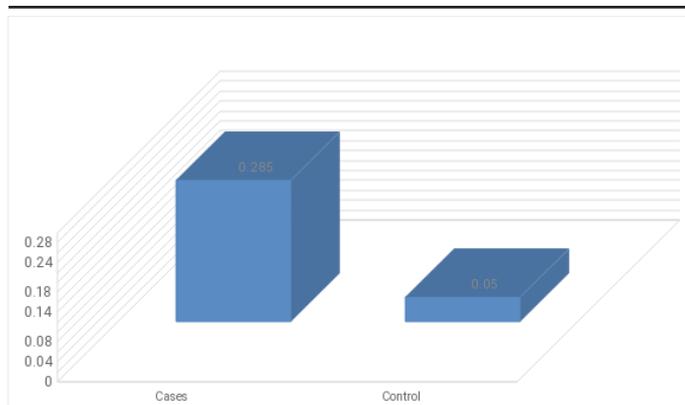
In wealthy nations, the prevalence of perinatal asphyxia is two cases per 1000 live births; but, in underdeveloped nations, where access to care for mothers and newborns may be restricted, the rate can reach ten times higher. Of the affected infants, 15–25% pass away during the neonatal stage, and up to 25% of the survivors suffer from lifelong neurologic impairments [16,17]. About 26.4% of newborns with NE who survived prior to the cooling era had moderate to severe neurodevelopmental damage, and an additional 14% had mild impairment. Although reported rates of cerebral palsy after NE vary, survivors of moderate to severe encephalopathy typically have rates between 10% and 13% [18,19].

The two most prevalent subtypes of cerebral palsy are dyskinetic CP and spastic quadriplegia, with prenatal hypoxia-ischemia at term accounting for 80% of dyskinetic CP cases [19]. Increased sensory disturbance occurs after hypoxic-ischaemic injury. It has been observed that individuals with other persistent neurological abnormalities have up to 17.1% hearing loss rates [20].

When combined with moderate to severe basal ganglia changes and severe white matter changes on MRI, the percentage of newborns with a diagnosis of Neonatal Encephalopathy who have abnormalities in some aspect of their visual function throughout the first year of life climbs to 100% [21].

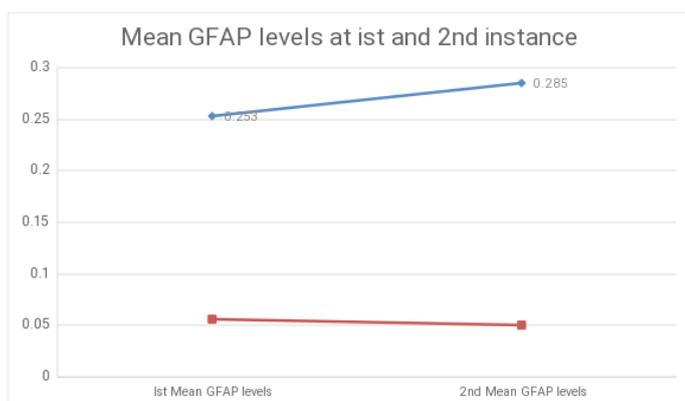
Only few therapeutic options are available at present to prevent the long term neurological effects of HIE including hypothermia, xenon, erythropoietin.

There is a need for establishing early and valid methods to characterize the risk for future neurodevelopmental outcome



**Figure 10:** Comparison of mean GFAP levels in Cases and Controls at 48 hours of life.

## Comparison between Mean GFAP levels at 1st (12 hours) and 2<sup>nd</sup> instance (48 hours)



**Figure 11**

Above graphical work represents the mean GFAP levels calculated at its and 2<sup>nd</sup> instance, the same reflects an increased levels of mean GFAP in cases at 2<sup>nd</sup> instance compared to its instance and a slightly decreased level in controls.

In our study, out of 35 cases, 7 patients died in neonatal period due to complications of HIE. The rest of the cases were followed up for neurodevelopmental outcome using HINE scoring.

### Correlation of HINE scoring and GFAP levels at 12 hours

Out of 16 patients with a normal neurodevelopment outcome as assessed by HINE, 14/16 (87.5%) had GFAP below a level of 0.24. All the patients, 12/12 (100%) with an adverse neurodevelopmental outcome had GFAP levels more than 0.24.

### Correlation of HINE scoring and GFAP levels at 48 hours

Out of 16 patients with a normal neurodevelopmental outcome as assessed by HINE, 15/16 (93.7%) had GFAP below a level of 0.24. All the patients, 12/12 (100%) with an adverse neurodevelopmental outcome had GFAP levels more than 0.24.

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which may help in early anticipation of infants at risk. Both therapeutic (hypothermia, erythropoietin, xenon etc.) and rehabilitative measures can then be targeted individually at high risk neonates.

A variety of methods have been tried to assess and characterize the neurological affliction and long term neurodevelopmental outcome including APGAR score and blood p<sup>H</sup>/ lactate, clinical examination, neuroimaging, EEG, heart rate variability and biomarkers. Each of these methods has its own advantages and disadvantages.

APGAR score and blood gases are usually the first available records in an asphyxiated newborn and have the advantage of easy availability and interpretation. However, both have been shown to have poor correlation with neurodevelopmental outcome in studies. In addition, APGAR score suffers from having inter-observer variability. A study showed that the sensitivity and the positive predictive value of low p<sup>H</sup> for adverse outcome were, respectively, 21 and 8%, of high lactate concentration 12 and 5%, and of low 5 minute Apgar score 12 and 19%. Metabolic acidosis determined in blood from the umbilical artery at birth is a poor predictor of perinatal brain damage [22].

The clinical examination of an asphyxiated infant requires high clinical skill and expertise and is subjected to inter observer variability. Standardised tests including Amiel Tison [23] and Dubowitz examination [24] have been developed to improve inter observer variability however, clinical examination in an ICU setting is often affected by sedation, mechanical ventilation and anti-convulsant.

Electroencephalography (EEG) and amplitude integrated EEG (aEEG) have both been shown to offer excellent predictive ability as early as 3-6 h following delivery [25]. Outcome is strongly linked with the severity of EEG abnormalities seen. EEG and aEEG abnormalities evolve over the first 72 h, and so the timing of the recording is crucial to interpretation [26].

However EEG needs resources, equipment to apply and clinical expertise to interpret.

Neuroimaging, mainly involving use of MRI brain and MRS is useful in showing specific patterns of brain injury and may detect even early changes. However, a normal MRI brain even though predictive of a good neurodevelopmental outcome does not completely rule out adverse outcomes as shown in a study which investigated the predictive value of a simple scoring system for perinatal MRI done in term infants after cooling for HIE and found lower scores were associated with fewer motor and tone problems but a normal MRI did not consistently equate to normal cognitive and language measures around 24 months of age [27].

Moreover, MRI Brain may not be feasible in a sick neonate who would require transportation to MRI unit and to be sedated for a prolonged period of time and often cannot be performed early enough when neuroprotective measures could be instituted.

Biomarkers are a novel concept to assess HIE and pilot studies have shown promise. A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention," according to the US National Institutes of Health Biomarkers Definitions Working Group [28].

The lack of measurable biomarkers that could assess the severity of injury, help with therapy triage, and provide prognostic information makes it difficult to manage newborns with Hypoxic-Ischemic Encephalopathy (HIE).

Quantitative biomarker testing that can identify preclinical lesions when routine brain imaging or monitoring is still silent would be a significant improvement in the management of newborns with suspected brain damage. Because of their short half-lives, biomarkers may be used to screen newborns for brain injury with high sensitivity and specificity, track the development of the injury and the response to therapy through repeated measures, and correlate with the degree of brain lesions later detected by MRI or ultrasound [29].

There is a dearth of research on the use, usefulness, and accuracy of biomarkers in HIE. Brain-specific proteins (S100B, ubiquitin carboxy-terminal hydrolase-L1 [UCH-L1], total tau, neuron-specific enolase), cytokines (interleukin [IL]-1 $\beta$ , IL-6, IL-8, IL-10, IL-12, IL-13, IFN- $\gamma$ , TNF- $\alpha$ , brain-derived neurotrophic factor [BDNF]), etc. are among the many biomarkers that have been proposed.

Our study aimed at evaluating one such biomarker known as Glial Fibrillary Acidic Protein (GFAP) in HIE.

GFAP is a key Intermediate Filament (IF) III protein responsible for the cytoskeleton structure of glia cells and for maintaining their mechanical strength, as well as supporting neighboring neurons and the Blood Brain Barrier (BBB) [30].

GFAP has been shown to increase in ischemia, trauma, intra cranial hemorrhage and stroke and cross the blood brain barrier to be detectable in the serum in measurable amounts. We selected GFAP in our study owing to commercial availability of the GFAP ELISA kit, quick and relative ease of calculating values.

We assessed the infants for neurodevelopmental followup using the Hammersmith Infant Neurological Examination due to certain salient features of the scale.

The Hammersmith Infant Neurological Examination (HINE) [31] has been suggested as one of the initial methods for the neurological evaluation to diagnose CP [10,32]. It is an easy-to-use, scoreable technique for assessing babies who are between the ages of two and twenty-four months. It consists of 26 items that evaluate several areas of neurological exams, including reflexes, posture, movements, tone, and cranial nerves [31]. The proforma includes pictures to facilitate recording as well as directions for completing each item separately. The HINE takes five to ten minutes to complete and is accessible to all physicians. Even with untrained staff, there has been good inter observer reliability recorded [10].

According to a systematic review of the literature, magnetic resonance imaging plus the General Movements Assessment or the Hammersmith Infant Neurological Examination is advised for neurodevelopmental assessment before the age of five months has been corrected; following this age, magnetic resonance imaging (where safe and practical), the Hammersmith Infant Neurological Examination, and the Developmental Assessment of Young Children are recommended [34].

While the HINE was first designed as a therapeutic tool, an optimality score was also created for scientific investigations. Based on the frequency distribution of scores in the normal population, the optimality score designates as optimal any score that is present in at least 90% of a cohort of typically develop-

ing, low-risk newborns evaluated at various ages. A global optimality score can be obtained by adding the individual scores, which range from 0 to 3, for each item. If every item receives a score of zero, the global score can be as low as 0 or as high as 78 (if every object receives a value of three or more).

When global scores are equal to or higher than 73 after nine to twelve months, or greater than 70 and 67 after six and three months, respectively, they are considered ideal [31,33]. A small number of tasks that accompany the development of some trunk control activities are the cause of the lower scores as age decreases [31,34]. Since its launch, the HINE has been suggested as an alternative for prognosis, diagnosis, and rehabilitation and utilised in various high- and low-risk groups, both for preterm and term-born children [35,36,37,38].

In our prospective case control study which was conducted over a period of two years with primary aim of evaluating the increase in GFAP levels as a prognostic tool for predicting the neurodevelopmental delay in patients with perinatal asphyxia induced HIE, we investigated a total of 70 neonates with 35 Cases and 35 Controls, there were 20 males and 15 females in Case group and 18 males and 17 females in Controls group (**Table 1**). The case and control groups were comparable with regards to sex distribution, mean birth weight, mean gestational age and the method and place of delivery (**Tables 1-5**).

Investigating the GFAP levels in Cases and Controls, we found the mean GFAP levels at 12 hours in Cases to be 0.253 while in Controls it was 0.056, which reflected a significant increase in GFAP levels from Controls with a p. value of <0.001 (**Table 6**).

In our study, the mean GFAP levels at 48 hours in Cases was 0.285 while in Controls it was 0.050, which again reflected a significant difference between Case and Control groups (**Table 7**).

Comparing the GFAP levels among subjects at 12 and 48 hours, we observed an **increase** in mean GFAP levels in Cases from 0.253 at 12 hours to 0.285 at 48 hours (**Figure 11**).

Our study showed that the mean levels of GFAP in Controls **decreased slightly** from the levels at 12 hours to 0.050 at 48 hours (**Figure 11**).

Among the cases, 7 patients died in neonatal period with complications of HIE.

We assessed the Neurodevelopmental outcome with the help of the HINE score at 3 months and at 6 months. We used regression analysis to compare GFAP levels and the corresponding HINE scoring.

In our study, assessing the relationship between GFAP levels and neurodevelopmental outcome (as defined by a HINE score <67 at 3 months and <70 at 6 months), we found that 12/12 (100%) patients with abnormal neurodevelopmental outcome had GFAP levels above 0.24 ng/mL at both 12 and 48 hours of life inferring that a cut off value of 0.24 ng/mL has 100% sensitivity in patients with abnormal neurodevelopmental outcome.

14/16 (87.5%) of patients with normal neurodevelopmental outcome at 3 and 6 months of life had GFAP levels below 0.24 ng/mL at 12 hours of life. Thereafter, at 48 hours of life we found that 15/16 (93.7%) of patients with normal neurodevelopmental outcome had GFAP levels below 0.24 ng/mL showing a specificity of 87.5% and 93.7% at 12 and 48 hours of life respectively (**Figure 12-15**).

Neurodevelopmental outcome at 3 and 6 months of life thus showed negative correlation with GFAP levels at both 12 and 48 hours of life.

Our study suggests that a GFAP cut off value of 0.24 ng/mL at 12 hours may predict with a sensitivity and specificity of 100% and 87.5% respectively abnormal outcome in Hypoxic Ischemic encephalopathy. At 48 hours the sensitivity of a GFAP cut off value of 0.24 ng/mL remains 100% but specificity increases to 93.7%.

Our findings were consistent with the study done by **Ennen et al.** who studied GFAP levels in neonates treated with whole body cooling compared to controls and found that none of the controls had a GFAP value above the 95<sup>th</sup> percentile versus 10/23(43.4%) cooled neonates. They had further documented abrupt elevations of GFAP the day after the 72 hour completion period was completed. Neonates with HIE and abnormal brain imaging had elevated GFAP levels compared to neonates with HIE and normal imaging. This study differed from our study in that therapeutic hypothermia used could have possibly affected the GFAP levels as evidenced by the abrupt elevations in GFAP after stopping hypothermia.

**Massaro et al** also found that glial fibrillary acidic protein was higher at 24 and 72 hours in babies with adverse outcomes compared with those with favorable outcome.

**Douglas Escobar et al** also demonstrated a rise in the concentration of GFAP with time over the 96 h measured in a study group comprised of neonates with HIE who were eligible for hypothermia according to a set predefined criteria based on gestational age & birth weights. They also demonstrated that serum concentrations of GFAP had a strong correlation to percent injury of the cortex at a time of 0-6 hrs of age and the percent injury of white matter and basal ganglia injury at 12 hrs of age. Neurodevelopmental outcome between 4.8 and 10 months of age also correlated to raised GFAP levels at 12 hrs.

**Blennow et al**, also observed that GFAP levels in asphyxiated groups was 5 times more than the reference group and that the levels increased with increasing severity of HIE.

**Chalak et al** also showed that GFAP levels correlated significantly with other indicators of birth depression and multiple organ dysfunction and that GFAP levels remained significantly higher in neonates with moderate to severe HIE compared to those with mild HIE at each time point after birth.

Our findings are in agreement with studies showing that high GFAP levels in immediate post natal period are associated with adverse neurodevelopmental outcomes later on.

The study was conducted at a single centre and with a limited number of patients, any inference in terms of results remain subject to further research with broader sample size.

### Summary

1. The present study has shown the following salient features;The mean serum GFAP level in the HIE group was 0.253 ng/mL while the mean GFAP level in the control group was 0.056 ng/mL at 12 hours of life. The difference between the two groups was statistically significant.(p< 0.001)
2. The mean serum GFAP level in the HIE group was 0.285 ng/mL while the mean GFAP level in the control group was 0.050 ng/mL at 48 hours of life. The difference between

the two groups was statistically significant. ( $p < 0.001$ )

3. Increased GFAP levels in postnatal period at both 12 and 48 hours of life were negatively correlated to neurodevelopmental outcome at 3 and 6 months of life. ( $p < 0.01$ )
4. A GFAP cut off value of 0.24 ng/mL at both 12 and 48 hours of life had 100% sensitivity in predicting adverse neurodevelopmental outcome at 3 and 6 months of age while having a specificity of 87.5% at 12 hours and 93.7% at 48 hours.

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