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Tau protein in Hypoxic Ischemic Encephalopathy

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ABSTRACT

Background: One of the most frequent causes of cerebral palsy (CP) and other neurological severe abnormalities in children is neonatal hypoxic-ischemic encephalopathy (HIE). 1.5 out of every 1000 live births result in neonatal HIE. The degree of brain maturation and the length and intensity of hypoxia determine the pattern of brain injury. Full-term neonates may present with different imaging findings than preterm neonates.

Objective: This work aimed to validate tau protein aggregates as an HIE biomarker.

Patients and methods: Forty newborns with suspected asphyxia were included in a case-control study that was conducted over 12 months, from February 2022 to February 2023 at the Neonatal Intensive Care Unit of Menoufia University Hospitals. **Results:** The level of tau protein in patients (163.00 ng/l) was considerably greater than in controls (120.75 ng/l). Tau protein's optimal cutoff point level as a diagnostic marker for HIE cases was > 94.80 ng/l, with a 94.7% specificity and 95.2% sensitivity at an AUC of 0.737.

Conclusion: Serum of tau protein levels within the first 24 hours of life can serve as a biomarker for both neurodevelopmental outcomes and an early diagnosis of neonatal HIE.

Keywords: Asphyxia, Encephalopathy, Hypoxic-ischemic, Neonates, Serum tau protein.

INTRODUCTION

One of the most frequent causes of CP and other serious neurological abnormalities in children is neonatal HIE. 1.5 out of every 1000 live births result in neonatal HIE. It is brought on by insufficient oxygen and blood flow to the brain, leading to diffusion or specific brain damage. The most important risk factor for HIE is asphyxia, which can result from several different circumstances. The degree of brain maturation and the length and intensity of hypoxia determine the pattern of brain injury. Full-term neonates may present different imaging findings than preterm neonates **(1).**

Neuroimaging methods include magnetic resonance imaging (MRI), computed tomography (CT), and ultrasound (US) aid in precisely locating, characterizing, and assessing the degree of brain damage. Due to their increased sensitivity in identifying acute brain injury, more recent imaging modalities including magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI) may play a part in prompt diagnosis and treatment **(2).**

The main goals of the treatment are to address the underlying issues and provide support. Research is being done on the potential of neuroprotective measures to reduce the degree of brain damage brought on by hypoxia-ischemia. The degree of the damage and the infant's gestational age determine the prognosis for HIE **(3).** The complex collection of cytoskeletal proteins keeps the central nervous system (CNS) function and anatomy. Microtubule-associated proteins (MAPs) are among the cytoskeletal proteins that seem particularly vulnerable to hypoxia or anoxia in the brain. As a result, MAP2 and other similar proteins are helpful, sensitive indicators of early agonal asphyxia. The human tau protein's natively unfolded protein structure, encoded by chromosome 17q21, contributes to its flexibility and capacity to stabilize functioning microtubules. Compared to other cytosolic proteins, its central structure, which consists of serines, threonines, aspartates, glutamates, lysines, arginines, prolines, and aromatics, is highly hydrophilic. Its N-terminal portion is primarily acidic, its middle region is rich in proline, and its C-terminal area is comparatively neutral**.** Tau possesses a transient secondary structure composed of poly-proline II helix, β-helices, and β-pleated sheets. Tau's overall hydrophilicity is attributed to its unfolded, denatured protein properties rather than its globular protein resemblance. Additionally, it can combine with other tau proteins to create aggregations**.** Another cytoskeletal protein, tau, has drawn increased interest recently because of its role in numerous neurological diseases. Tau is crucial for microtubule construction and stabilization in healthy brains. Additionally, tau may support neurite outgrowth, stability throughout brain development, interactions with the actin cytoskeleton, and signal transduction pathways**.** Tau is discovered to be aggregated aberrant filaments, such as neurofibrillary tangles, localized in the somatodendritic compartments of cells, as opposed to its typical location in axons in the brains of individuals with tauopathic diseases. Certain tauopathies and disease-causing mutations have different effects on the distribution and ultra-structural morphology of tangles in the brain **(4)**.

Parkinson's disease patients also have protein aggregation, primarily in the midbrain's substantia nigra. According to immunohistochemical research, Lewy bodies' protein aggregates develop in the substantia nigra in part due to aberrantly phosphorylated tau proteins. This region's dopaminergic neuronal neurodegeneration may be mediated by Lewy bodies **(5).** This work aims to validate tau protein aggregates as an HIE biomarker.

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PATIENTS AND METHODS

Forty newborns with suspected asphyxia were included in a case-control study that was conducted over twelve months, from February 2022 to February 2023, at the Neonatal Intensive Care Unit of Menoufia University Hospitals.

- **Group 1 (Cases)**, according to the Leven stage, 40 newborns were suspected of being asphyxiated upon admission to our NICU. These infants met three or more of the clinical and biochemical criteria [6]. Asphyxia was defined as the necessity for positive pressure breathing, an Apgar score of fewer than three at the fifth minute, a pH of less than 7.0, or a base deficit of less than 12 in cord or venous blood obtained from babies within 60 minutes of delivery (> 3 min) [7].
- **Group 2 (Control group):** included 30 healthy neonates (no signs of fetal distress, pH more than 7.2, and Apgar scores at 1 and 5 min more than 7).

Exclusion criteria: All newborns with any deformity, intrauterine growth retardation, hemolytic or cardiac diseases, congenital or perinatal infections such as chorioamnionitis, and a family history of cognitive impairment.

Sample size estimation: During the study period from February 2022 to February 2023, a similar number of neonates with HIE were recruited from the Neonatal Intensive Care Units of Menoufia University Hospital and El-Bagour Central Hospital to form the cluster sample.

All patients were subjected to the following:

- History taking and data collection that included the personal history of the neonate as name, gestational age, sex, age (days) at the time of diagnosis of suspected HIE, maternal history, prenatal history, natal history, and post-natal history.

- General examination included gestational age estimation using the new Ballard score [8], body weight, and head circumference. **Vital signs** are assessed for temperature, heart rate, and respiratory rate.

- Systemic Examination: Included chest, cardiac, and neurological examination (within the first 24 h after birth and for 7 d to classify HIE according to the criteria of Sarnat and Sarnat staging [9]. Evaluation of the neonatal reflexes, convulsions, activity, posture, tone, and quantity of anticonvulsant medications.

Routine investigations such as The Sysmex Xp 300 (Sysmex Company, Germany) provided a complete blood count that included hemoglobin (Hb), white blood cells (WBCs), and platelets. The Cobas 6000 analyzer (c501 module) (Roche Diagnostics- GmbH, D-68305 Mannheim, Germany) is used to measure the liver function test (ALT, AST). Kidney function test using CX9 Beckman Colter auto analysis (serum urea and creatinine). Venous blood gases (PH-PCO2-HCO3-BE) were measured with Medica easy stat and ABL 800 instrumentation. Sodium, potassium, and calcium electrolyte analysis was conducted using the PathFAST Analyzer (Willstatter Str. 30, 405-49 Dusseldorf, Germany). The Uright TD-4279 Glugometer was used to measure blood sugar randomly (blood glucose monitoring system).

Specific investigation: Tau protein was assessed using Human Tau proteinate (PROTEIN ELISA Kit (201-12-7416).

Ethics approval: Every procedure was carried out in compliance with the Institutional Committee's Ethical Guidelines. Menoufia University Faculty of Medicine's Ethical Committee approved the study. After explaining the purpose and methodology of the study to each participant or their parents, signed informed consents were obtained from all participants or their parents. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

SPSS version 25.0 was used on an IBM-compatible personal computer to gather, tabulate, and statistically analyze the data (MEDCALC V.19.6.1 and IBM SPSS Statistics for Windows, v. 25.0, Armonk, NY, IBM Corp). Student t-test (t), Mann-Whitney test (U), Chi-square test (Fisher or Monte Carlo), multiple regression analysis, binary logistic regression and Kruskall-Wallis test (K), Pearson correlation and The ROC (receiver operating characteristic) curve were used. Statistical significance was established at $p \leq 0.05$.

RESULTS

Figure (1) depicts a flowchart of the 95 newborns at Menoufia University Hospitals who had HIE as part of the research cohort. Twenty-five were removed (four patients failed the sample preservation procedure, eight failed sample centrifugations because the sample amount was insufficient, sample hemolysis, or sample adhesion to the tube), and thirteen patients did not match the inclusion criteria. Seventy patients were included in the study. As shown in figure (1), they were split into two groups: The cases group (n = 40) and the control group (n = 30). GA was substantially lower in cases (34.73±2.75) than in controls (37.47±1.38) (P<0.0001). The post-natal age of cases was found to be considerably greater (13.83±11.44) compared to controls (6.60±6.20) (P=0.001). However, there was no discernible variation in sex between the groups under investigation (P=0.562). Additionally, there was a significant difference (P<0.05) in height and weight between the patients and controls (47.45±3.10 cm, 2.61±0.72 kg and 49.60±6.20 cm, 3.23±0.46 kg). Furthermore, BMI was substantially lower in patients (10.80) compared to controls (12.96) (P<0.001). When comparing the head circumference of patients (33.05±1.99 cm) to controls (34.77±1.07 cm) with a significant difference (P<0.001), (Table 1).

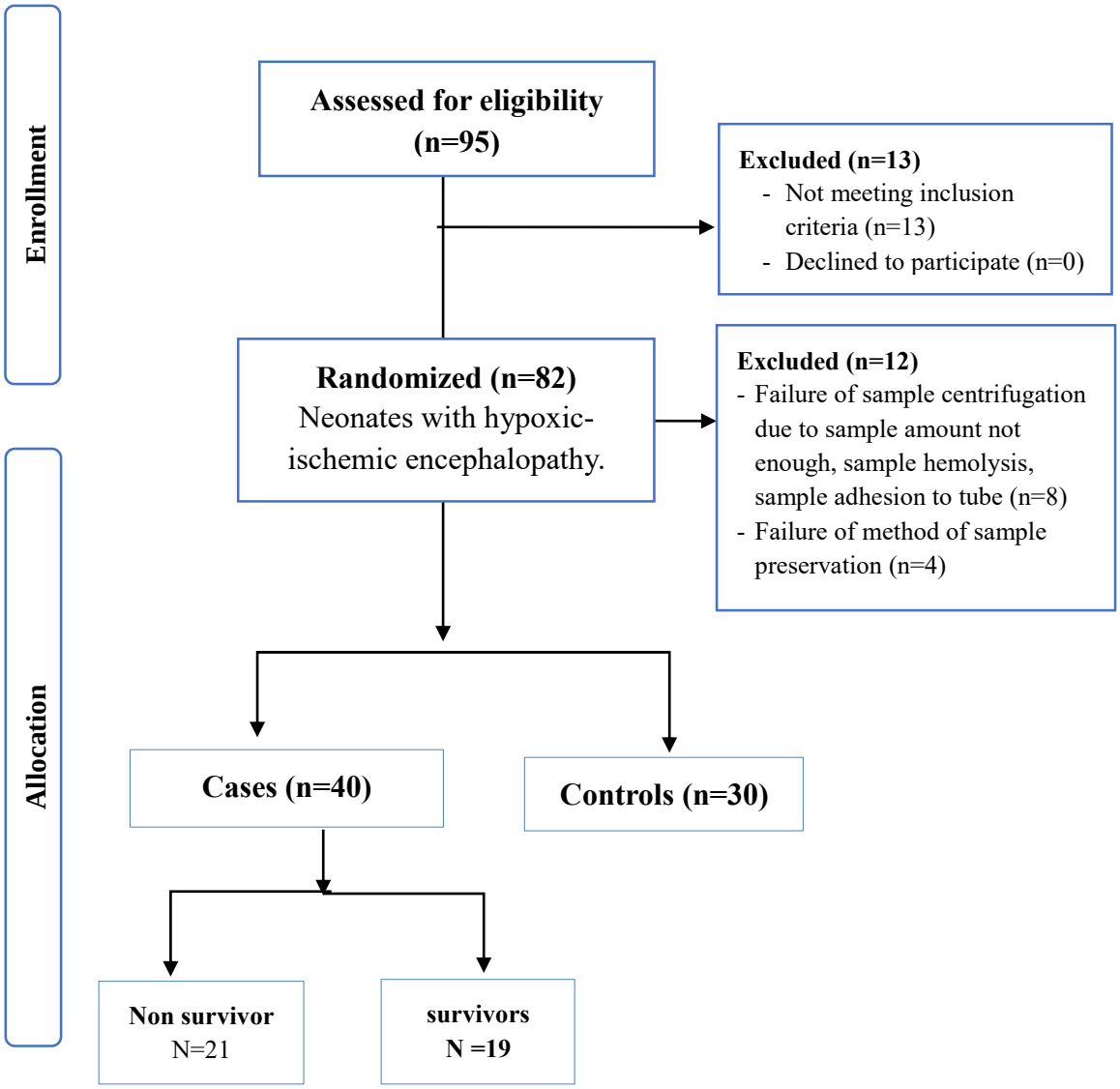

Fig. (1): Flowchart of neonates with HIE.

Table (1): Personal history of the neonates among the studied groups

Variables	Cases (N= 40)		Control (N= 30)		*t*	*P-value*
	N	%	N	%		
GA (weeks) Mean ± SD	34.73±2.75		37.47±1.38		5.459	**<0.001***
Sex Male	23	57.5	19	63.3	X^2=0.337	0.562
Female	17	42.5	11	36.7		
Age post-natal (days) Mean ± SD	13.83±11.44		6.60±6.20		3.388	**0.001***
Weight (kg) Mean ± SD	2.61±0.72		3.23±0.46		4.360	**<0.001***
Height (cm) Mean ± SD	47.45±3.10		49.60±1.75		3.668	**0.001***
BMI (kg/m2) Median (IQR)	10.80 (7.65-15.92)		12.96 (9.31-16.92)		**U=** 278.500	**<0.001***
Head circumference (cm) Mean ± SD	33.05±1.99		34.77±1.07		t=4.632	**<0.001***

GA: Gestational age, BMI: Body mass index, t: Independent t test, U: Mann Whitney u test, X^2: Chi square test, *Significant

Furthermore, there was a significant difference in hemoglobin between the patients (13.25±2.89 g/dl) and controls (15.71±2.65 g/dl) (P<0.001). Also, compared to controls (273.50, 7.37, 19.35, 8.75, 4.70), patients (190.00, 7.33, 18.00, 8.40, 4.10) had significantly reduced PLTs, PH, HCO_3, Ca total, and Ca ionized (P<0.05). However, there was a substantial difference in tau protein (163.00) between patients and controls (120.75) (P<0.001). However, WBCs, urea, creatinine, ALT, AST, PCO_2, Na, K, and random blood sugar did not significantly differ across the groups under investigation (P>0.05) **(Table 2).**

Table (2): Laboratory Investigations among the studied groups

Variables	Cases (N= 40)	Control (N= 30)	*U*	*P-value*
	Median (IQR)	Median (IQR)		
Hemoglobin (g/L) Mean ± SD	13.25±2.89	15.71±2.65	t=3.704	**<0.001***
WBCs (10^9/L	9.70 (4.50-37.00)	8.90 (4.50-22.60)	497.500	0.224
PLTs (10^9/L)	190.00 (27.00-616.00)	273.50 (156.00-560.00)	319.000	**0.001***
Urea (mg/dl), mean ±SD	35.03±8.70	33.87±7.12	t=0.338	0.736
Creatinine (mg/dl) Mean ± SD	0.94±0.22	0.85±0.16	t=1.247	0.217
ALT (IU/L)	27.00 (14.00-90.00)	28.00 (15.00-47.00)	543.500	0.502
AST (IU/L)	41.00 (23.00-913.00)	38.50 (21.00-56.00)	454.000	0.083
PH	7.33 (7.18-7.46)	7.37 (7.30-7.49)	344.500	**0.002***
PCO_2 (mmHg)	41.00 (17.00-67.00)	39.50 (25.00-49.00)	515.000	0.312
HCO_3 (mEq/L)	18.00 (11.50-26.50)	19.35 (15.60-26.00)	400.000	**0.018***
Na (mmol/L)	138.00 (126.00-148.00)	138.00 (127.00-145.00)	589.500	0.901
Ca Total (mg/dl)	8.40 (5.70-10.00)	8.75 (7.70-10.20)	375.500	**0.008***
Ca Ionized (mg/dl)	4.10 (3.00-5.10)	4.70 (3.80-5.30)	274.500	**<0.001***
K (mmol/L)	4.50 (3.20-8.70)	4.20 (3.50-5.50)	536.000	0.446
Tau Protein (ng/L)	163.00 (90.57-1207.00)	120.75 (88.70-173.10)	238.500	**<0.001***
RBCs (mg/dl), mean ± SD	105.33±25.31	96.20±13.85	t=1.154	0.253

Median and IQR: non-parametric test, t: independent test, U: Mann Whitney u test, *Significant. WBCs: white blood cells, Ca: calcium, PH: phosphorous, Na: sodium, k: potassium, Ca: Calcium, PCO2: partial pressure of carbon dioxide, HCO3: bicarbonate, PLT: platelets, ALT: Alanine transaminase-test, AST: aspartate aminotransferase, RBS: Random blood sugar.

The tau protein and sex did not significantly correlate (P>0.05). Concurrently, a significant correlation was observed (P<0.05) between tau protein and the following parameters: natal and postnatal history, consciousness level, activity, tone, convulsions, transcranial ultrasound, severity, and death. While, tau protein was significantly higher among severe patients than moderate and mild patients (P<0.001) (Table 3).

Table (3): TAU protein levels in relation to the studied variables among the studied cases (n=40)

Variables	TAU protein		*U*	*P-value*
	No.	Mean± SD		
Sex				
Male	23	413.98±93.26	179.000	0.605
Female	17	442.15±49.47		
Natal history				
Normal vaginal delivery	18	358.21±37.46	**H =** 3.592	0.166
Cesarean section	20	427.74±35.02		
Obstructed labor	2	1031.87±27.67		
Postnatal history				
RDS	10	178.22±12.43		
Asphyxia	6	1007.20±157.62		
Pneumonia	9	146.44±5.24		
Sepsis	5	179.32±1.84	**H =** 27.025	**0.001***
Hyoxia	4	966.96±15.53		
Malignant TTN	2	124.50±5.56		
Hemorrhagic disease of newborn	1	163.00±0.001		
Aspiration	2	1037.81±100.11		
Ischmia	1	670.80±0.001		
Consciousness level				
Normal	19	156.45±6.99	65.000	**<0.001***
Disturbed	21	671.13±40.30		
Activity				
Active	12	127.35±23.53	**H =** 13.833	**0.001***
Hypoactive	26	574.04±47.51		
Moderate	2	306.45±52.96		
Tone				
Normal tone	13	138.24±5.29	54.000	**<0.001***
Hypotonia	27	565.52±41.55		
Convulsions				
No	26	284.82±24.64	71.000	**0.002***
Yes	14	690.06±45.67		
Transcranial U\S (HIE)				
Grade 1	12	122.42±21.59	**H=** 29.838	**<0.001***
Grade 2	12	172.43±5.75		
Grade 3	7	613.65±34.76		
Grade 4	9	1025.83±13.01		
Severity				
Mild	12	123.04±20.79	**H =** 28.615	**<0.001***
Moderate	12	176.25±6.80		
Severe	16	845.50±17.74		
Mortality				
Survivor	19	243.73±28.77	105.000	**0.010***
Non survivor	21	592.16±41.64		

TTN: Transient tachypnea of the newborn, RDS: Respiratory distress syndrome, U: Mann Whitney you test, H: Kruskall Wallis H test, *Significant.

Furthermore, tau protein levels, salt, potassium, and creatinine were found to be the most significant factors influencing the death rate among the patients under study, according to logistic regression analysis (P<0.05). Other examined parameters, however, did not demonstrate a statistically significant impact on the study patients' mortality rate (P>0.05) (Table 4).

Table (4): Logistic regression analysis for the studied parameters affecting mortality among the studied cases

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI[B]	
	B	Std. Error	Beta			Lower	Upper
GA (weeks)	0.000	0.077	0.001	0.003	0.998	-0.164	0.165
Age (post-natal) (days)	0.008	0.010	0.174	0.734	0.474	-0.014	0.030
BMI (kg/m2)	0.035	0.049	0.141	0.726	0.479	-0.068	0.139
Head circumference (cm)	-0.079	0.083	-0.305	-0.953	0.356	-0.256	0.098
Temperature ©	0.149	0.294	0.098	0.507	0.620	-0.477	0.775
Heart rate (bp.)	0.008	0.005	0.247	1.554	0.141	-0.003	0.020
Respiratory rate (bm.)	0.003	0.009	0.071	0.347	0.733	-0.016	0.022
Hemoglobin (g/L)	0.005	0.034	0.030	0.149	0.884	-0.068	0.078
WBCs (^9/L)	-0.012	0.016	-0.204	-0.748	0.466	-0.045	0.021
P6LTs (10^9/L)	0.001	0.001	0.184	0.924	0.370	-0.001	0.002
Urea (mg/dl)	0.013	0.008	0.386	1.742	0.102	-0.003	0.029
Creatinine (mg/dl)	1.012	0.360	0.765	2.809	**0.013***	0.244	1.780
ALT (IU/L)	-0.005	0.006	-0.199	-0.777	0.449	-0.018	0.009
AST (IU/L)	-0.002	0.001	-0.473	-1.375	0.189	-0.004	0.001
PH	-1.655	1.475	-0.208	-1.121	0.280	-4.799	1.490
Pco2 (mmHg)	0.013	0.009	0.304	1.437	0.171	-0.006	0.031
Hco3 (mEq/L)	0.014	0.030	0.103	0.465	0.649	-0.049	0.077
Na (mmol/L)	1.890	0.018	0.980	3.485	**0.013***	0.046	1.985
Ca total (mg/dl)	0.185	0.144	0.276	1.285	0.218	-0.122	0.491
Ca ionized (mg/dl)	-0.232	0.224	-0.193	-1.037	0.316	-0.709	0.245
K (mmol/L)	0.922	0.143	0.429	1.554	**0.041***	0.0820	1.526
Random blood sugar (mg/dl)	-0.002	0.002	-0.154	-0.789	0.442	-0.007	0.003
Tau protein (ng/L)	0.001	0.0001	0.711	3.682	**0.002***	0.700	2.001

GA: gestational age, BMI: body mass index, WBCs: white blood cells, Ca: calcium, PH: phosphorous, Na: sodium, k: potassium, Ca: Calcium, PCO2: partial pressure of carbon dioxide, HCO3: bicarbonate, PLT: platelets, ALT: Alanine transaminase-test, AST: aspartate aminotransferase, CI: Confidence Interval *Significant

The optimal cutoff point level of tau protein as a diagnostic marker for HIE cases, according to ROC curve analysis, was > 94.80 ng/L, with a sensitivity of 95.2% and a specificity of 94.7% at an AUC of 0.737 **(**Table 5, Figure 2).

Table (5): ROC curve of Tau protein levels as a diagnostic marker for HIE patients

Test Result Variable(s)	AUC	Std. Error	Sig.	Ses.	Spac.	Cutoff value	95% C I	
							Lower	Upper
Tau protein (ng/L)	0.737	0.082	**0.01***	95.2	94.7	>94.800	0.58	0.90

CI: Confidence Interval *Significant

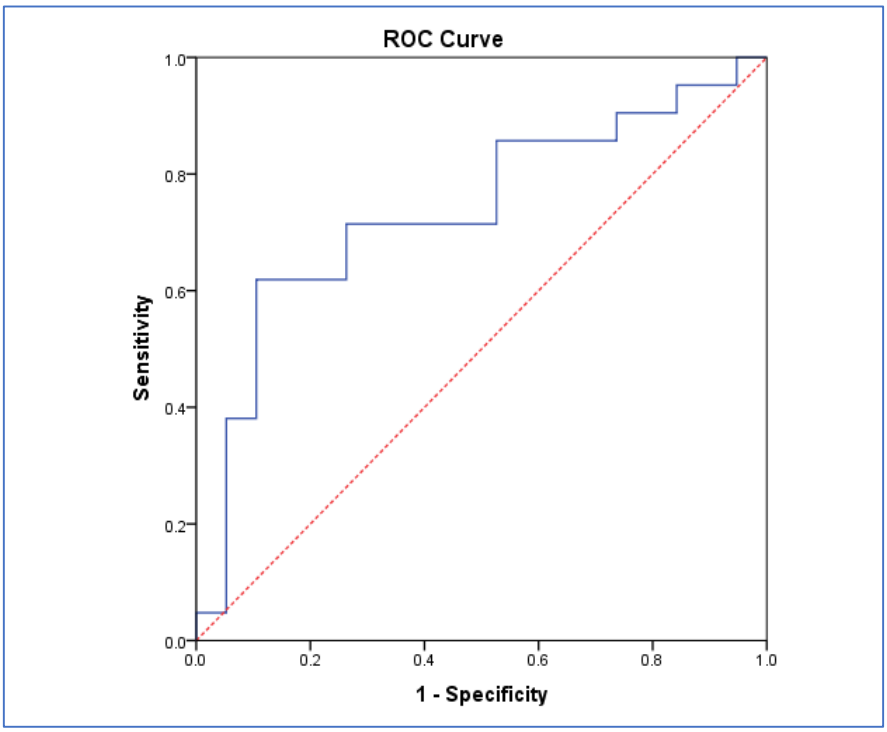

Fig. (2): ROC curve of Tau protein as a biomarker for HIE patients.

Tau protein levels were found to significantly increase the estimated median mortality rate among severe patients at hazard risks of 1.953 (95% CI: 868.949-1155.051) compared to mild patients at risks of 0.912 (95 CI: 124.35-149.85) and moderate patients at risks of 0.800 (95% CI, 145.0319-267.911), with a highly significant level reached (log-rank test, P<0.001), according to Kaplan-Meier survival curves (Table 6, figures 3 & 4).

Table (6): Means and Medians for mortality rate using Kaplan–Meier survival analysis among the studied cases

Responding	Mean				Median			
	Estimate Hazard	Std. Error	95% CI (Tau protein)		Estimate	Std. Error	95% CI (Tau protein)	
			Lower	Upper			Lower	Upper
Mild	0.9480	4.893	122.326	141.507	0.912	6.505	124.350	149.850
Moderate	0.7830	20.887	171.855	253.731	0.800	19.330	145.319	267.911
Severe	1.9160	70.883	767.563	1045.425	1.953	72.985	868.949	1155.051
Overall	0.8793	82.801	535.504	860.082	0.967	180.347	436.600	1143.560
Log Rank (Mantel-Cox)							X^2=20.36, P<0.001*	
Breslow (Generalized Wilcoxon)							X^2=15.86, P<0.001*	
Tarone-Ware							X^2=18.09, P<0.001*	

CI: Confidence Interval

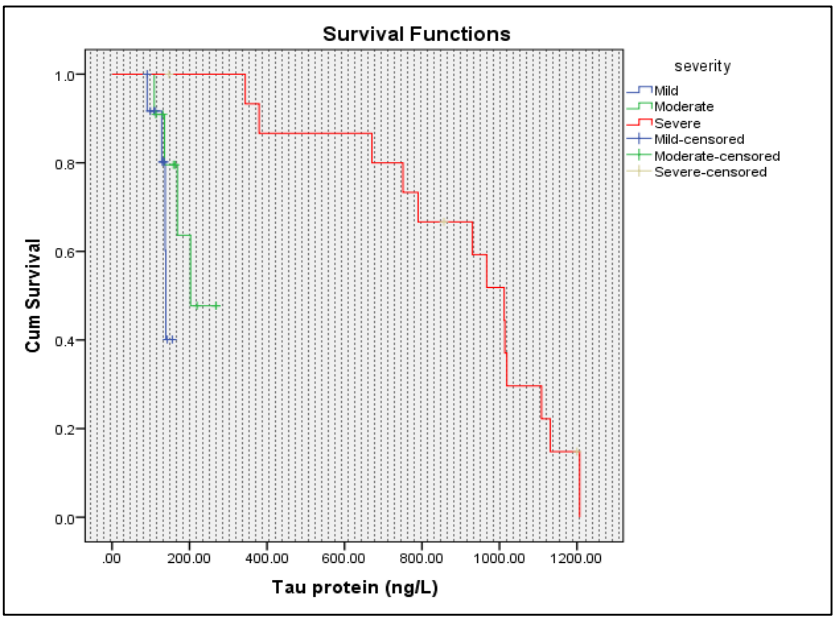

Fig. (3): Survival function according to Tau protein levels using Kaplan–Meier survival analysis.

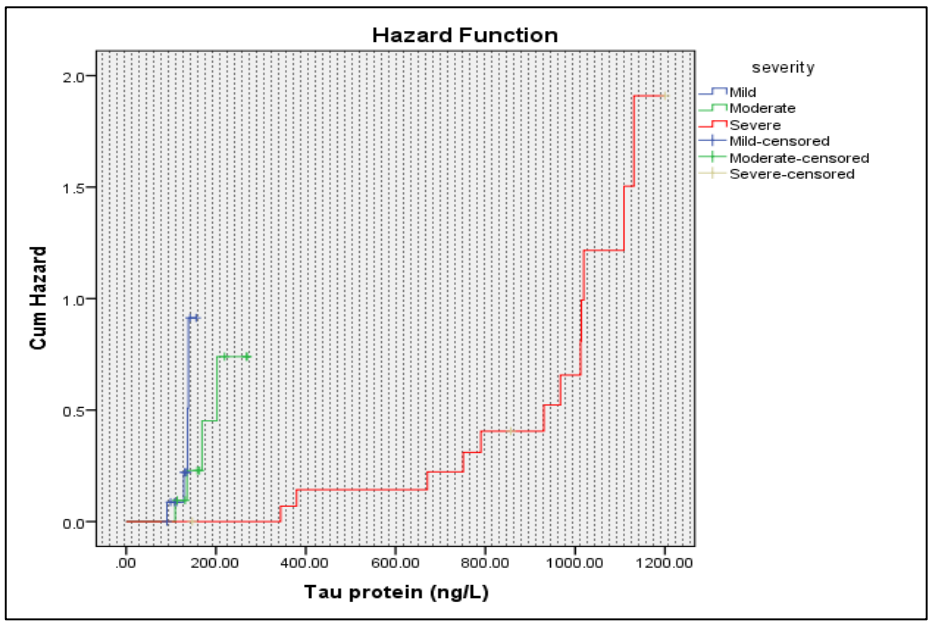

Fig. (4): Hazard function according to Tau protein levels using Kaplan–Meier survival analysis.

DISCUSSION

Neonatal HIE, mainly brought on by prenatal hypoxia, typically results in brain damage, primarily exhibited as neurological system dysfunction and physical development retardation, as well as possible death. Maternal factors, which include pregnancy complications like advanced age, infections, and abnormalities in amniotic fluid, as well as other significant factors like pre- and post-mature delivery, low birth weight, and birth injury, are the main factors influencing the occurrence of HIE [(10)].

It is difficult to obtain an early diagnosis of HIE because of the abnormality in embryonic brain development and the absence of recognizable clinical symptoms as well as the incidence of missed diagnoses and clinical misdiagnoses. According to **Lv *et al.*** [(11)], there is a strong correlation between neuronal loss and cognitive decline and the levels of microtubule-associated tau protein in plasma. Mild hypothermia is one of the most promising treatments for HIE among available choices; its therapeutic mechanism is yet unknown [(12)]. Two commonly used tools in the efficacy evaluation of HIE treatments are the Gesell development scale and the NBNA rating.

Our research revealed that whereas age post-natal was significantly higher in cases than in controls and that GA was much lower in cases than in controls, there was no significant difference in sex between the groups under investigation. Similarly, **Futrakul *et al.*** [(13)] discovered that there were noticeably more male infants in the cases (OR 2.3). One risk factor for HIE (OR 4.8) is male gender. It is yet unclear how significant this

discovery is. On the other hand, **Sitthivuddhi *et al.*** [14] found no evidence of a significant correlation between birth weight and HIE. There was a marginally higher risk (OR 4.7) for small gestational age (SGA) newborns, but this difference was not statistically significant. The current study's small sample size may have led to the unfavorable connection between SGA and increasing risk of HIE.

Otherwise, **Liu and Mccullough** [15] found that there was no statistically significant difference in the two groups' sex distribution, birth weight, mode of delivery, or gestational age. Infants in the HIE group exhibited larger percentages of amniotic fluid stained with meconium and statistically substantially lower Apgar scores than controls. Oliguria (46.43%), respiratory failure (39.29%), convulsion (35.71%), gastric hemorrhage (25%), hypocalcemia (60.71%), and other sequelae were associated with asphyxia. A bedside cranial ultrasonography performed on 11 newborns (39.29%) after 2-3 days of birth showed indications of cerebral edema. Five newborns (all I degree; three in the moderate and two in the mod severe HIE groups; no difference between the two groups) experienced periventricular hemorrhage. Also, **Butt *et al.*** [16] found no evidence that the gestational age of the newborn was a significant risk factor.

The results of this study showed that cases had significantly lower height and weight than controls, as well as significantly lower BMI and head circumference. Occipital-frontal head circumference (OFHC), or just head circumference, has only a clinical connection (16% vs. 10%) with post-natal variables, as would be predicted. However, **Hayes** [17] and **Lee** [18] have reported a significant statistical association with HIE.

In our study, in terms of the mother's history of diabetes mellitus, our study found no discernible differences between the groups under investigation. While, there were notable differences between the groups under study in terms of prenatal history (placenta abruption), natal history (obstructed labor), and postnatal history, the most common post-natal history was respiratory distress syndrome (RDS (10 cases, or 25%), followed by pneumonia (8 cases, or 20%), cord around the neck (7 cases, or 17.5%), and aspiration (milk and meconium) (8 cases, or 20%). **Liljestrom *et al.*** [19] found no correlation between a history of seizures or maternal diabetes mellitus in the same research. Risk factors and incidence of HIE can change over time and amongst groups [20]. Placental abruption could be a more frequent sentinel event in preterm birth, explaining both the beginning of preterm birth and HIE. **Butt *et al.*** [16] discovered that the mode of delivery did not seem to matter. The fact that surgical and instrumental births would have been performed by qualified medical professionals in their research population, providing a benefit, maybe the reason why they were not linked to birth asphyxia in the current study group. In contrast, a Ugandan study by **Kaye** [21] discovered a strong correlation between birth hypoxia and Cesarean sections (OR 2.36) and vacuum extractions (OR 2.16).

In contrast, **Butt *et al.*** [16] found a strong association has been discovered between HIE and the individual making the delivery. Compared to neonates in the control group, infants with HIE had a higher likelihood of being delivered by inexperienced delivery attendants (OR 3.2, 95% CI 1.9-5.3). The distribution method was yet another element that affected the prevalence of HIE. Compared to vaginally delivered babies, neonates born via Cesarean section (CS) had odds of developing HIE that were more than seven times higher. This aligns with other research projects in Northern Ethiopia, Istanbul, and Cameroon [22]**.** This result might be explained by the fact that CS is frequently carried out as an emergency procedure in cases of fetal distress or other issues that could jeopardize the fetus's oxygen supply. CS, however, may also have some detrimental effects on the infant, including iatrogenic preterm, transitory tachypnea, and respiratory distress syndrome [23]**.** Thus, proper monitoring and care should be given to minimize the occurrence of HIE, and CS should be saved for situations where there is a clear indication and benefit for the mother and the fetus.

In our study, hemoglobin levels in patients were noticeably lower than in controls. Additionally, there was a substantial difference between patients and controls in PLTs, PH, HCO_3, Ca Total, and Ca Ionized. On the other hand, whereas tau protein was considerably greater in cases (163.00) compared to controls, there was no significant difference seen in WBCs, urea, creatinine, ALT, AST, PCO_2, Na, K, and random blood sugar among the examined groups. On the other hand, **El-Gamasy and Alarabawy** [24] discovered that there was no statistically significant variation between the groups for HB, platelets, WBCs, and serum K. Furthermore, **Liu *et al.*** [15] discovered no variation in serum tau protein levels between the HIE and control groups. There was no discernible variation in the dynamic monitoring of serum tau protein in varying degrees of HIE. It could be concluded that there is no evidence of early up-regulation of tau protein in HIE. In their data, neonates with HIE had lower blood calcium concentrations, pH, BE, HCO_3, CK, CK-MB, LDH, and MYO, and higher concentrations of WBC, CK, and CK-MB, indicating that multiple systems were injured following asphyxia. However, no correlation was found between these biochemical parameters and the degree of encephalopathy.

Lv *et al.* [25] study indicated that the levels of serum tau protein in 40 cases (1013 pg/ml [538.04–1190.42]) were found to be considerably higher than those in the control group (106.41 pg/ml [64.55–154.71], p =.0001). When compared to the umbilical cord around the neck with hypoxic-ischemic encephalopathy UCAN-HIE group (892.78 pg/ml

[538.04–1179.50], p =.0149), the serum tau protein levels in the placental abruption with hypoxic-ischemic encephalopathy PA-HIE group (1024.46 pg/ml [657.88–1190.42]) were significantly higher. The PA-HIE group's development quotient score (67.0 [47.0–90.0]) was considerably lower than the UCAN-HIE group's (81.5 [52.6–100.0]) (p =.0028). In comparison with the UCAN-HIE group (22.73%), the PA-HIE group's component ratio of neurodevelopmental retardation (44.45%) was considerably higher (X2 = 13.3138, p =.0013).

Our study showed that at an AUC of 0.737, the optimal cutoff point level of tau protein was > 94.80 ng/L, resulting in a sensitivity of 95.2% and a specificity of 94.7% as a diagnostic marker for HIE cases. Similarly, tau was found to be more accurate in predicting neurological prognosis at 48 and 72 hours following the arrest than NSE as reported by **Torbenson *et al.* [26],** who evaluated tau in the targeted temperature management (TTM) trial cohort. The best accuracy was noted at 72 hours. In their investigation, 7.9 ng/l with a good 71% sensitivity was the cutoff value for reaching 95% specificity at 72 hours. This is consistent with the threshold found by **Humaloja *et al.*** [27] (7.75 pg/ml, sensitivity 88%). Although the differences were not statistically significant, the AUROC values of tau in this investigation were more significant than the AUROC values of NSE at 48 and 72 hours. Neonatal HIE severity and prognosis can be assessed using biomarkers for brain injury found in serum or cerebrospinal fluid [11].

In hypothermia or medication intervention approaches, these signs are even thought of as therapy effect indices. After neuronal damage, tau protein, a microtubule protein of central neurons, breaks free from the microtubule and enters the blood and cerebrospinal fluid. As a result, tau protein is a sign of brain damage. Tau protein is a very specialized component found in neurons. Furthermore, after adult cerebral ischemia, clinical trials have demonstrated a high correlation between tau protein levels and the infarct volume and the severity of the disease [28]. Accordingly, the degree of neuronal injury can be directly indicated by blood tau protein levels [29, 30]. According to our earlier research, newborns with HIE had significantly greater serum tau protein levels than healthy neonates, and those with severe HIE had significantly higher levels than those with mild HIE.

CONCLUSION

Serum tau protein level within 24 hours after birth can be utilized as a marker for neurodevelopmental outcomes and an early diagnosis of neonatal HIE, with severe instances being more likely to be diagnosed than mild or moderate cases. The acquired data provide a new understanding of the role of the tau protein gene in controlling delayed neuronal death in the ischemic hippocampal region. Ultimately, these results provide additional insight into the long-term effects of cerebral ischemia on the onset of Alzheimer's disease.

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REFERENCES

1. **Barta H, Jermendy A, Kovacs L *et al.* (2022):** Predictive performance, and metabolite dynamics of proton MR spectroscopy in neonatal hypoxic–ischemic encephalopathy. Pediatric Research, 91 (3): 581-89.
2. **Yıldız E, Ekici B, Tatlı B (2017):** Neonatal hypoxic ischemic encephalopathy: an update on disease pathogenesis and treatment. Expert Review of Neurotherapeutics, 17 (5): 449-59.
3. **Salama M, Mohamed W (2016):** Tau protein as a biomarker for asphyxia: A possible forensic tool? Applied & Translational Genomics, 9: 20-22.
4. **Wang D, Huang X, Yan L *et al.* (2021):** The Structure Biology of Tau and Clue for Aggregation Inhibitor Design. The Protein Journals, 40 (5): 656-68.
5. **Villamil-Ortiz J, Cardona-Gomez G (2015):** Comparative analysis of autophagy and tauopathy related markers in cerebral ischemia and Alzheimer's disease animal models. Frontiers in Aging Neuroscience, 7: 84. doi: 10.3389/fnagi.2015.00084.
6. **Fredricks T, Gibson C, Essien F *et al.* (2017):** Therapeutic hypothermia to treat a newborn with perinatal hypoxic-ischemic encephalopathy. The Journal of the American Osteopathic Associations, 117 (117): 393-8.
7. **Ahearne C, Boylan G, Murray D (2016):** Short- and long-term prognosis in perinatal asphyxia: An update. World Journal of Clinical Pediatrics, 5 (1): 67-73.
8. **Ballard J, Khoury J, Wedig K *et al.* (1991):** New Ballard Score, expanded to include extremely premature infants. J Pediatr., 119 (3): 417-23.
9. **Sarnat H, Sarnat M (1976):** Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol., 33 (10): 696-705.
10. **Xu J, Gang Q, Hao P *et al.* (2016):** Pathological and magnetic resonance imaging findings in a neonatal Tibet minipig model of hypoxic-ischemic encephalopathy. Nan Fang Yi Ke Da Xue Xue Bao., 36: 705–709.
11. **Lv H, Wang Q, Wu S *et al.* (2015):** Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clin Chim Acta., 450: 282–297.
12. **Elbahtiti A, Aly NY, Abo-Lila R *et al.* (2016):** Therapeutic hypothermia for infants with hypoxic ischemic encephalopathy: a five years' single center experience in Kuwait. J Neonatal Perinatal Med., 9: 179–185.
13. **Futrakul S, Praisuwanna P, Thaitumyanon P (2006):** Risk factors for hypoxic-ishemic encephalopathy in asphyxiated newborn infants. J Med Assoc Thai., 89: 322-28.
14. **Sitthivuddhi Futrakul M, Praisuwanna P, Thaitumyanon P (2006):** Risk factors for hypoxic-ischemic encephalopathy in asphyxiated newborn infants. J Med Assoc Thai., 89 (3): 322-8.

15. **Liu F, Mccullough L (2013):** Inflammatory responses in hypoxic ischemic encephalopathy. Acta Pharmacologica Sinica., 34 (9): 1121-30.
16. **Butt T, Farooqui R, Khan M (2008):** Risk factors for hypoxic ischemic encephalopathy in children. J Coll Physicians Surg Pak., 18 (7): 428-32.
17. **Hayes B, McGravy C, Mulvany S (2013):** A case control study of hypoxic ischemic encephalopathy infants > 36 weeks gestation. Am J Obstet Gynecol., 209 (1): 29. doi: 10.1016/j.ajog.2013.03.023.
18. **Lee AC (2008):** The risk factors for neonatal mortality due to birth asphyxia in Southern Nepal. Pediatrics, 121(5): 1381–1390.
19. **Liljestrom L, Wikstrom A, Agren J *et al.* (2018):** Antepartum risk factors for moderate to severe neonatal hypoxic ischemic encephalopathy: a Swedish national cohort study. Acta obstetricia et gynecologica Scandinavica, 97 (5): 615-23.
20. **Kurinczuk J, White-Koning M, Badawi N (2010):** Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev., 86: 329–38.
21. **Kaye D (2003):** Antenatal and intrapartum risk factors for birth asphyxia among emergency obstetric referrals in Mulago hospital, Kampala, Uganda. East Afr Med J., 80: 140–3.
22. **Bayih W, Birhane B, Belay D *et al.* (2021):** The state of birth asphyxia in Ethiopia: An umbrella review of systematic review and meta-analysis reports, 2020. Heliyon, 7: e08128. doi: 10.1016/j.heliyon.2021.e08128.
23. **Umbwiyeneza J (2017):** Effect of mode of delivery on outcomes of full-term neonates admitted in a tertiary Hospital of Kigali. University of Rwanda Digital Repository, 35: 29.doi: http://hdl.handle.net/123456789/408
24. **El-Gamasy M, Alarabawy R (2018):** Relation of Serum Creatinine to Sarnat Scoring and Brain Computerized Tomography of Neonates with Hypoxic Ischemic Encephalopathy. A Single-Center Experience. Journal of Pediatric Neurosciences, 13 (4): 437-442.
25. **Lv H, Wang Q, Chen H *et al.* (2020):** Study on serum Tau protein level and neurodevelopmental outcome of placental abruption with neonatal hypoxic-ischemic encephalopathy. The Journal of Maternal-Fetal & Neonatal Medicine, 33 (23): 3887-93.
26. **Torbenson V, Tolcher M, Nesbitt K *et al.* (2017):** Intrapartum factors associated with neonatal hypoxic ischemic encephalopathy: a case-controlled study. BMC Pregnancy Childbirth, 17 (1): 415. doi: 10.1186/s12884-017-1610-3.
27. **Humaloja J, Lähde M, Ashton N *et al.* (2022):** GFAp and tau protein as predictors of neurological outcome after out-of-hospital cardiac arrest: A post hoc analysis of the COMACARE trial. Resuscitation, 170: 141-149.
28. **Kiyani A, Khushdil A, Ehsan A (2014):** Perinatal factors leading to birth asphyxia among term newborns in a tertiary care hospital. Iran J Pediatr., 24: 637–42
29. **Nanavati T, Seemaladinne N, Regier M *et al.* (2015):** Can we predict functional outcome in neonates with hypoxic ischemic encephalopathy by the combination of neuroimaging and electroencephalography. Pediatrics & Neonatology, 56 (5): 307-16.
30. **Logitharajah P, Rutherford M, Cowan F (2009):** Hypoxic-ischemic encephalopathy in preterm infants: antecedent factors, brain imaging, and outcome. Pediatric Research, 66 (2): 222-29.
31.