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Circulating Dickkopf-1 and Cranial Ultrasound Correlation in Newborn with Hypoxic- Ischemic Encephalopathy

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Abstract: Hypoxic-Ischemic Encephalopathy (HIE) is the most devastating brain disorder that leads to cerebral palsy in neonates. Cranial imaging in HIE is crucial and comprises four different types of imaging for accurate diagnosis. Cranial ultrasound is one of the imaging techniques that is commonly used, particularly, in low income countries and can be used without anesthesia. There is no gold standard for the diagnosis of HIE however, in recent years, studies have found a notable link between the increase in serum Dickkopf-1, a potent inhibitor of the Wnt signaling pathway, and deterioration of HIE cases. The detection of Dickkopf-1 in high concentration could be associated with deterioration in brain function and proliferation of cells leading to severe disabilities and death. Therefore, this study aimed at comparing serum Dickkopf-1 in neonates with HIE with control group. As well, to correlate different grades of HIE, serum Dickkopf-1 and cranial ultrasound changes in HIE neonates. A case-control study was conducted in the neonatal intensive care units (NICU) in Fayoum general hospital, from May 2018 to August 2019. Forty full term neonates of ≥ 37 gestational weeks were assessed regarding serum Dickkopf-1, blood components, resistive index, and cranial ultrasound changes in both HIE neonates and controls. Dickkopf-1 was found positively correlated with poor neurodevelopmental outcome, increased resistive index, and high mortality among HIE neonates. In addition, major cranial ultrasound changes were associated with higher serum levels of Dickkopf-1. This study results recommend the use of Dickkopf-1 as a predictor with ultrasonography for good prognosis and diagnosis of HIE neonates.

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1. Introduction:

Hypoxic-Ischemic Encephalopathy (HIE) occurs to full term neonates during the prenatal, intrapartum or postnatal period (1) causing shortage of blood circulation to the brain (2,3). HIE is one of the most predominant causes of cerebral palsy (CP), mental retardation, and epilepsy (2,4). Around 60% of infants with HIE will die or have severe mental disability by the age of two. Unfortunately, the development of the obstetric care could not render the occurrence of hypoxic- ischemic events. Nevertheless, medical care aims at minimizing the consequences of brain injury and other severe neurologic symptoms in those neonates. Incidence of HIE was estimated by 2 to 9 cases per 1000 live births worldwide, 2.3 – 26.5 per 1,000 live births in developing countries(5) and about 1.5 to 2.5 per 1000 live births in developed countries (2). Studies have demonstrated the occurrence of HIE to neonates who are beyond 35 to 36 weeks(3,6). The exact pathophysiology of HIE is not fully known however, the degree of the insult depends mainly on the brutality of low blood circulated to the brain and the degree of brain maturation(3).

Several factors could contribute to the occurrence of HIE although not always identified. For instance, antecedents include cord prolapse, uterine rupture, abruptio placenta, placenta previa, maternal hypotension, breech presentation, or shoulder dystonia (2). Others could owe to inadequate placental perfusion and impaired gaseous exchange due to fetal factors i.e. fetal bradycardia, fetal thrombosis, and fetal hemorrhage, or maternal factors i.e. preeclampsia, severe anemia, asthma and chronic vascular disease. Postnatal HIE could occur in neonatal pulmonary failure such as severe hyaline membrane disease, meconium aspiration syndrome, pneumonia, or congenital cardiac disease (7).

In HIE, neonates are presented with low Apgar scores, fetal distress, presence of meconium stained, neonatal encephalopathy, and neonatal acidemia i.e. pH < 7.0 or base deficit ≥ 12 mmol/L (2,8). In the first 24 hours of a neonate's life, he/she may develop

symptomatic apnea or seizures with abnormal electroencephalographic (EEG) (2).

The prediction of HIE is critical and should start as soon as possible to minimize consequences. Although, MRI is the gold standard scanning technique used in HIE, cranial US has been found to be feasible as MRI, particularly, when MRI is not available i.e. in developing countries (Annink et al., 2020; Kumar et al., 2016). Cranial Ultrasound (US) is a convenient, affordable, and noninvasive screening method of the unstable neonate at the bedside. Nevertheless, parenchymal abnormalities i.e. periventricular Leukomalacia (PVL) and cerebral edema, identified at US are often non-specific. Sonography is subtle for possible hemorrhage, PVL, and hydrocephalus. In addition, sonography is operator dependent and less sensitive to structural abnormalities in the cerebral convexity and in the brainstem. For cerebral perfusion, Doppler interrogation along with the assessment of resistive index (RI) provide additional information in staging process of HIE. The development of intracranial hemorrhage or diffuse cerebral edema and loss of forward diastolic flow results in increased RI and is an early sign for poor outcome.

Human Dickkopf-1 (DKK-1) is a member of the Dickkopf gene family which secrets a protein involved in embryonic development. Dickkopf-1 is a potent inhibitor of the Wnt signaling pathway, which plays a critical role in cell patterning, proliferation, and fate determination during embryogenesis (9) and hippocampal function through life (10). In the embryonic brain, Wnt signaling induces self-renewal of radial glia progenitors and differentiation, but not the proliferation of intermediate progenitors (11). Excessive expression of Dkk1 severely reduces neurogenesis in the developing hippocampus and has been related to neurodegenerative processes associated with Alzheimer's disease or brain ischemia(10,12). These findings are thought to predict HIE with abnormalities on cranial ultrasound and could be correlated with serum Dickkopf-1.

Consequently, this study aimed at comparing serum Dickkopf-1 in neonates with HIE and normal neonates. Also, to correlate different grades of hypoxic ischemic encephalopathy, serum dickkopf-1 and cranial ultrasound changes in HIE neonates.

2. Methodology and patients:

I. Study design:

A case-control study was carried out in the neonatal intensive care units (NICU) in Fayoum general hospital, from May 2018 to August 2019 after approval from ethics committee (no. 45)-faculty of medicine- Fayoum university in January/ 2018 – (D143) and a written informed consent was obtained from all caregivers/parents of the involved neonates in accordance with the Declaration of Helsinki. The study included 40 full term (≥ 37 gestational weeks) neonates and they were divided into two equal groups.

II. Patients selection:

Inclusion criteria:

Controls:

20 full term healthy neonates have a gestational age of 37 weeks and older, delivered either by cesarean section or vaginally.

Cases:

20 full term HIE neonates, 37 weeks of gestational age or older, with hypoxic ischemic encephalopathy satisfied inclusion criteria, delivered either by cesarean section or vaginally. Have been diagnosed according to Sarnat and Sarnat classification(13) and fulfilled at least two of the clinical findings listed below:

1. Apgar score <5 at 5 min (14).
2. Fetal acidosis, (as umbilical cord pH below 7.2 or in neonatal blood samples obtained on the first day of life).
3. Fetal distress (such as abnormal fetal heart rate and meconium stained amniotic fluid).
4. Fetal asphyxia required resuscitation as need for assisted ventilation (mask/balloon or intubation).
5. Encephalopathy (lethargy/stupor, hypotonia and abnormal reflexes including an absent or weak suck).
6. Presence of convulsions in the first 24 h of life
7. Multiple organ dysfunctions (encephalopathy and the involvement of at least one organ).
8. Agreed written informed consent from caregivers/parents.

Exclusion criteria:

Infants were excluded from the study if they met any of the following conditions:

1. Cases with severe sepsis or congenital brain infection.
2. Full term newborn with major congenital or chromosomal anomalies.
3. Inborn errors of metabolism.
4. Infants with sepsis who were excluded depending on clinical and laboratory findings.

III. Diagnosis of HIE in our neonates

1. Complete medical history: full maternal and prenatal, natal, postnasal history of diseases and medications was obtained for all cases.
2. Thorough clinical examination: full neurological examination was done during the first 24 hour focusing on signs of encephalopathy:

A. According to Sarnat and Saranat classification of HIE(13).

B. Apgar score <5 at 5 minutes (14).

IV. Laboratory methods

The laboratory investigations include, Complete blood count (CBC): using automated cell counter (coulter), Mindry BC 3600 China also, measurement of serum DKK-1 level: using Human Dickkopf-1 ELISA Kit NEW TEST SUPPORT CO. Toronto, Canada.

A. Sampling: 4ml of venous blood was drawn from each neonate of both groups and was divided as follow: 2 ml blood was added to EDTA tube for CBC and 2 ml blood was added to another plain tube; stand for 30 minutes, centrifuged at 3000 rpm for 10 minutes, supernatant serum was separated in isolated Eppendorf tube and preserved at -20º C until time of assay of DKK-1.
B. Serum Dickkopf1 level assay: Assays for DKK-1 were withdrawn at the first 24 hour of age using ELISA and then was measure spectrophotometrically at 450 nm.

V. Radiological Assessment

Using Doppler sonography, imaging of the medial cerebral arteries was bilaterally conducted through the right and left temporal bone on axial planes. In all subjects, peak systolic velocity, diastolic velocity and RI were measured in middle cerebral artery.

RI is defined as peak systolic velocity minus end diastolic velocity divided by peak systolic velocity. Normally in term infants, RI in middle cerebral artery is 0.7. Abnormal RI is defined as 0.55 or less measured at least in one artery(15). Each neonate with HIE had brain scan by cranial ultrasound examinations upon discharge.

VI. Statistics

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package V. 22 (SPSS Inc, USA). For quantitative data, the mean, median, standard deviation (SD), and Inter-quartile range (IQR) were calculated. Kolmogorov-Smirnov test (KS) test was performed as a test of normality. If variables were not normally distributed, either Mann-Whitney-U test or Kruskal-Wallis test was used while comparing between any two or three groups, respectively. Otherwise, Independent t-test was used. Qualitative data were presented as number and percentages, chi square ($\chi 2$) was used as a test of significance. Spearman correlation was run to identify relation between Dickkopf-1 and several parameters among HIE cases. ROC curve was used to determine the cut-off point in which highest sensitivity and specificity of Dickkopf-1 as predictors in differentiating between different classifications. For interpretation of significance, significance was adopted at $P \leq 0.05$.

3. Results:

In table 1, no significant difference was associated with gestational age, sex, mode of delivery and birth weight among HIE neonates and controls. In addition, cases showed 15 males and 5 females. While control group showed 11 males and 9 females.

Table (1): Demographic data of the studied groups:

Variable	Cases(N=20)		Controls(N=20)		P-value
	Mean±SD		Mean±SD		
Gestational age	38.5±1.3		38.3±0.9		0.582
Birth Weight	3.2±0.4		3.2±0.3		0.789
Variable	**N**	**%**	**N**	**%**	**P-value**
Sex					
Male	15	75.0%	11	55.0%	0.185
Female	5	25.0%	9	45.0%	
Mode of delivery					
Vaginal	12	60.0%	10	50.0%	0.525
CS	8	40.0%	10	50.0%	

CS: Cesarean Section

In table 2, a total of 7 neonates or 35% showed mild grade HIE, 55% showed moderate, 10% showed severe grade. Regarding oxygen support facility, 65% needed nasal oxygen, while the remaining were ventilated. Regarding convulsion, 85% had convulsion accordingly, 23.5% needed one anticonvulsant, 9 (52.9%) needed two medication and 23.5% needed three medication to control convulsions.

Table (2): Clinical characteristics of HIE cases (Group I):

Variable	N	%
Grade		
Mild	7	35.0%
Moderate	11	55.0%
Severe	2	10.0%
Oxygen support		
Ventilated	13	65.0%
Oxygen support	7	35.0%
Convulsions		
Yes	17	85.0%
No	3	15.0%
Anticonvulsant		
1	4	23.5%
2	9	52.9%
3	4	23.5%

Regarding cranial ultrasonographic among the studied HIE neonates, a total of 15.4% showed increased echogenicity of parenchyma, 7.7% showed increased echogenicity of thalami, 23.1% showed prominent ventricles, 15.4% showed cystic changes, 15.4% showed abnormal RI, **table 3**.

Table (3): Cranial ultrasonographic findings of cases

Variable	N	%
Increased echogenicity of parenchyma	2	15.4%
Increased echogenicity of thalami	1	7.7%
Prominent ventricles	3	23.1%
Cystic changes	2	15.4%
Abnormal RI	2	15.4%
Variable	**Mean**	**SD**
	Median	**IQR**
RI	0.7	0.08
	0.72	(0.67-0.76)

RI: Resistive Index; IQR: Interquartile Range

Neurological changes and mortality were assessed where 35% died before discharge. Among the survived diseased neonates, 61.5% had abnormal neurological examination and 38.5% had normal neurological examination as shown in **table 4**.

Apgar score was significantly lower in all HIE neonates at both < 1 and < 5 min than controls as shown in **table 5**. Comparison between Hemoglobin (HB) level, White Blood count (WBCs) and platelets between HIE neonates and controls showed a significant difference as shown in **table 6**. A significant difference as regard to serum DKK-1 level in favor of HIE neonates was found with almost double the range (658.5-781.5) in comparison with control group, as shown in **table 7**.

Table (4): Mortality and neurological outcome in the cases:

	N	%
Mortality		
Died	7	35.0%
Survived	13	65.0%
Neurological outcome		
Abnormal	8	61.5%
Normal	5	38.5%

Table (5): Apgar score at first day for all studied newborn

Variable		Cases (N=20)		Controls (N=20)		P-value
		Mean	SD	Mean	SD	
Apgar sore at 1 min	<5	20	100.0	0	0.0	<0.0001*
	>5	0	0.0	20	100.0	
Apgar score at 5 min	<5	20	100.0	0	0.0	<0.0001*
	>5	0	0.0	20	100.0	

*Significant

Table (6): Laboratory parameters comparison between cases and controls:

Variable	Cases (N=20)	Controls (N=20)	P-value
	Mean±SD Range	Mean±SD Range	
HB(gm/dl)	13.9±1.5 (9.8-16.2)	16.6±1.2 (15.4-18.7)	<0.0001*
WBCs× (10^3/mm^3)	15.1±2.6 (11.2-19)	17.9±2.5 (13.9-24)	0.001*
Platelets× (10^3/mm^3)	185.3±60.7 (55-285)	301±70.7 (182-400)	<0.0001*

HB: Hemoglobin; WBC: white blood cells.*Significant

Table (7): Serum Dickkopf -1 comparison between cases and controls:

Variable	Cases (N=20)	Controls (N=20)	P-value
	Mean±SD Range	Mean±SD Range	
Dickkopf-1 (pg/ml)	745.35±125.93 (658.5-781.5)	340.71±83.25 (303.7-386.5)	<0.0001*

*Significant

Regarding association between serum Dickkopf-1 and other HIE neonatal parameters, a positive strong correlation between serum Dickkopf-1 and HIE grades was detected. A strong negative correlation between serum Dickkopf-1 and WBCs, HB, and platelets were detected(P<0.0001). Insignificant weak correlation between RI and serum Dickkopf-1 was detected, **table 8**.

Increase in HIE severity was significant with the increase in serum Dickkopf-1 (P<0.001). Ventilated neonates had higher serum Dickkopf-1 than those on oxygen support (P<0.001). In addition, serum Dickkopf-1 was significant in neonates who had convulsion and with those who took more than 1 drug, **table 9**.

There was a significant result between serum DKK-1 level and ultrasonographic changes in the form of prominent ventricles with no statistical significance regarding other forms of cranial changes, **table 10**.

Table (8): Correlation between Dickkopf-1 and several parameters among cases:

	Dickkopf-1	
	r	**P-value**
Gestational age	0.250	0.288
Birth weight	0.194	0.412
HIE grades	0.875	**<0.0001***
WBCs	-0.757	**<0.0001***
HB	-0.802	**<0.0001***
Platelets	-0.890	**<0.0001***
RI	0.058	0.850

HIE: hypoxic ischemic encephalopathy; HB: Hemoglobin; WBC: white blood cells, RI: resistive index. *Significant

Table (9): Relation between Dickkopf-1 and clinical characteristics among cases:

	Dickkopf-1	**P-value**
	Mean±SD	
Grade		
Mild	654.57±15.08	**0.001***
Moderate	745.55±50.16	
Severe	1062±128.69	
Oxygen support		
Ventilated	789.08±136.65	**0.001***
Oxygen support	664.14±32.16	
Convulsions		
Yes	763.06±128.84	**0.007***
No	645±9.54	
Anticonvulsant		
1	661.75±15.28	**0.002***
2	731.22±42.01	
3	936±164.26	

*Significant

Death was found significantly higher in neonates with higher serum level of DKK-1, **table 11**.

Regarding demographic data and mortality in HIE neonates, no significant difference was found between mortality rates and; gestational age, sex, and birth weight. Only a significant difference was found as regard to delivery mode, **table 12**.

Death was significantly higher in severely HIE cases (28.6%), cases on ventilation **(**100.0%), and on 3 prescribed anticonvulsant drugs, while insignificant higher mortality was associated with patients with convulsion, **table 13**.

Finally, using the ROC curve, the prediction of mortality among cases using serum DKK-1 was 85.7% specific and 100% sensitivity. In addition, the predicted differences between cases and controls are 100% specific and sensitive according to the ROC curve, as shown in figure (1).

Table (10): Relation between Dickkopf-1 and U/S findings among cases:

	Dickkopf		P-value
	Median	IQR	
increased echogenicity of parenchyma			
Positive	730.5	(729-732)	0.103
Negative	662	(648-704)	
increased echogenicity of thalami			
Positive	750	(750-750)	0.154
Negative	667	(651-711)	
prominent ventricles			
Positive	732	(729-750)	**0.007***
Negative	658.5	(648-683)	
cystic changes			
Positive	730.5	(729-732)	0.103
Negative	662	(648-704)	

*Significant

Table (11): Relation between Dickkopf-1 level and mortality:

Variable	Died (N=7)	Survived (N=13)	P-value
	Mean±SD Rang	Mean±SD Rang	
Dickkopf-1 (pg/mol)	860±153.81 (774-1062)	683.62±28.52 (654-718)	**<0.0001***

*Significant

Table (12): Relation between mortality and basic characteristics among cases:

Variable	Died (N=7)		Survived (N=13)		P-value
	Mean±SD		Mean±SD		
Gestational age	39.29±1.5		38.08±1.04		0.088
Birth Weight	3.33±0.28		3.08±0.42		0.189
Variable	**N**	**%**	**N**	**%**	**P-value**
Sex					
Male	6	85.7%	9	69.2%	0.613
Female	1	14.3%	4	30.8%	
Mode of delivery					
Vaginal	7	100.0%	5	38.5%	**0.015***
CS	0	0.0%	8	61.5%	

CS: Cesarean section.*Significant

Table (13): Relation between mortality and clinical characteristics among cases:

	Died (N=7)		Survived (N=13)		
Variable	**N**	**%**	**N**	**%**	**P-value#**
Grade					
Mild	0	0.0%	7	53.8%	**0.018***
Moderate	5	71.4%	6	46.2%	
Severe	2	28.6%	0	0.0%	
Oxygen support					
Ventilated	7	100.0%	6	46.2%	**0.044***
Oxygen support	0	0.0%	7	53.8%	
Convulsions					
Yes	7	100.0%	10	76.9%	0.521
No	0	0.0%	3	23.1%	
Anticonvulsant					
1	0	0.0%	4	40.0%	**0.013***
2	3	42.9%	6	60.0%	
3	4	57.1%	0	0.0%	

*Significant

Table (14): Accuracy of Dickkopf-1:

Condition	AUC	P-value	Cut-off point	Sensitivity %	Specificity %
Deforestation cases from control	1.000	**<0.0001***	551	100.0	100.0
Prediction of mortality among cases	0.954	**0.001***	762	85.7	100.0

*Significant

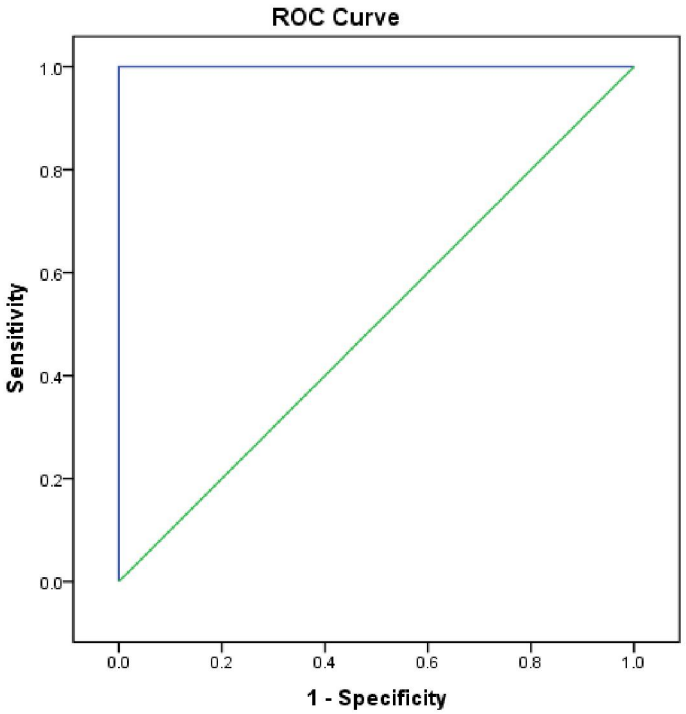

Figure (1) ROC curve of Dickkopf-1 for differentiating cases from controls ROC curves

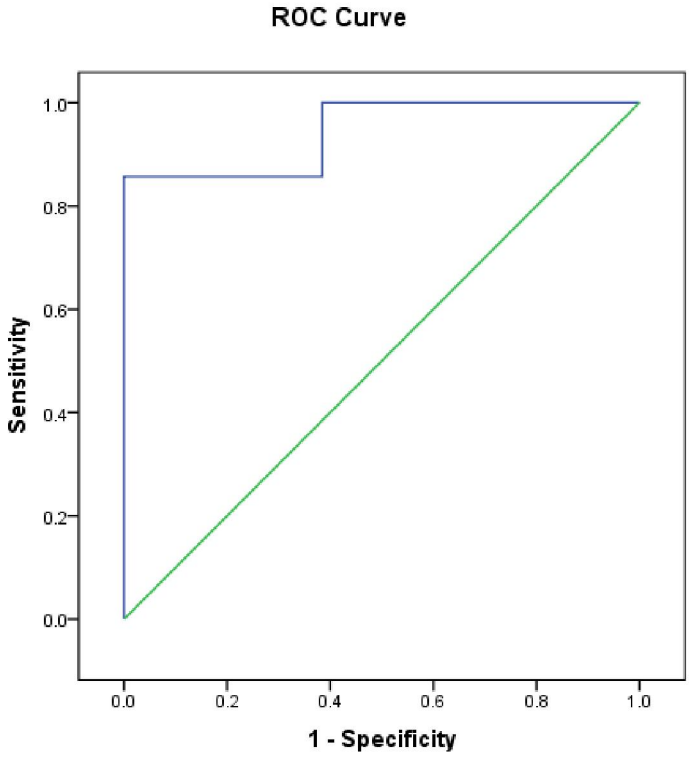

Figure (6b) ROC curve of Dickkopf-1 for predicting mortality among cases

4. Discussion:

HIE is the most catastrophic brain condition that could jeopardies neonatal development. Asphyxia is the most predominant risk factor in HIE. Both pattern and level of brain injury in HIE rely on degree of hypoxia and brain maturation where the findings in full-term neonates (>36 weeks of gestation) will definitely differ from those <36 weeks of gestation (7,16). In HIE, the complexed signs and symptoms mask the ability to determine appropriate timing of treatment. As well, brain hypoxia following HIE is complex and deteriorates over time. However, the pathologic stages of HIE come in two phases: primary energy failure and secondary energy failure(2). This comes along with the findings in this study where cranial US showed significant changes accompanied by HIE. 55% of the cases had moderate level of HIE where most of them (23.1%) suffered prominent ventricular changes followed by equal percentage of cystic changes, increased echogenicity of parenchyma and abnormal RI. Additionally, Salas study has demonstrated edema and hyperechogenicity in the perirolandic region using cranial ultrasonography in HIE neonates (17) while Banos study has concluded the detection of hydrocephalus and cystic periventricular white matter using US (7).

Regarding RI, 15.4% of the cases had abnormal RI with an IQR between 0.67-0.76. Diagnosis using US and RI was reported to measure Cerebral Vascular Dynamics (CVD) as well as the integrity of cerebral autoregulation in HIE (17,18) which goes with our study finding. Unfortunately, cranial US cannot detect cortical lesions, marked interobserver variability and operator dependency (7). Low cerebral RI was found to differentiate asphyxiated neonates and its subsequent neurodevelopmental impairment (19). As well, a study by Liu and colleagues has demonstrated that the velocity of cerebral blood flow was proportionally influenced by the decrease or increase in RI. The study has revealed that an RI of < 0.50 or > 0.90 was associated with severe cases while RI > 1.0 would be found in brain death (18). Similarly, duplex US with RI were both assessed to provide a measure of the severity of HIE. The study has concluded that RI $\leq$ 0.55 was found to be highly associated with either death or severe disability(17). This comes in the line with our study results where 35.0% of the cases who had moderate ranges of RI have died while 61.5% suffered abnormal neurological symptoms. Salas study has also added that US is becoming increasingly effective in determining the pattern, timing, and extent of injury in Hypoxic-Ischemic

Injury (HII), as well as it is easily portable, no need for sedation, with relatively low cost when compared to MRI (17).

In our study, prominent ventricles were found significantly differ in patients with higher serum Dickkopf -1. Nevertheless, other ultrasonographic changes were found insignificantly differ regarding serum Dickkopf-1. Although fewer study could linked the concentration of DKK1 and brain changes in HIE, Dickkopf-1 was found strongly correlated with patients with brain trauma(20,21), particularly, HIE (22–24). Interestingly, in our study, serum Dickkopf -1 was found strongly associated with high HIE grades with nearly double the ranges in the controls (r=0.875, P <0.0001). The increase in serum Dickopf-1 has been studied in many literature to predict traumatic brain disorder whether after brain injury or stroke (21) or in HIE (22–24). Those studies have revealed a strong correlation between circulating Dickkopf -1 and predicting brain encephalopathy as well as mortality in patients with severe HIE which matches our study finding.

Sixty-five percent of HIE neonates in our study were on ventilation. While 85.0% of the HIE neonates suffered convulsion in which, 52.9% had to take 2 anticonvulsants for managing epilepsy. It is well known that HIE causes 60% of all neonatal seizures(25) particularly, those with moderate to severe HIE (26,27). Reports have concluded that seizures could be observed in HIE neonates aged from 12-24 hrs (25,28). Unfortunately, in 50% of the cases, seizures become subtle to be noticed (29) especially in premature neonates (28). It is evident that seizures can cause further damage to the brain of middle to mild HIE neonates (25). Similarly, Dizon study has also mentioned the direct association between encephalopathy grades and seizure which agrees with our study results whereas 85% of the moderately HIE cases had convulsions and required treatment with more than one antiepileptic drug (AED). Glass and colleagues have reported that neonates with severe seizures had the worst neurodevelopmental outcomes with low Full-Scale Intelligence Quotient (FSIQ)(30) which consequently agrees with our findings.

According to our study results, HIE cases showed lower Apgar score compared to the control group whereas 100% of the cases recorded <5 Apgar score at 5 min and at 1 min. This goes in the line with the results by Dizon et al where 98% of HIE neonates showed Apgar scores ≤5 at 5, 10 and 15 min of life, severe encephalopathy, resuscitation in the delivery room, with the use of vasopressors in neonates presented with seizures (31). Similarly, low Apgars (<5) were reported to be associated with HIE (32–34) and cerebral palsy (33).

In addition, correlation between Dickkopf -1 and other biomarkers namely; HB, platelets, and WBCs showed a significant negative correlation (p<0.0001). For instance, the mean hemoglobin concentration was significantly lower in HIE neonates (13.9gm/dl) in comparison to the control group (16.6gm/dl); this result was in agreement with (23,35). Also, the mean platelets count was significantly lower in HIE neonates(185.3×103/mm3) in comparison to the control group(301×103/mm3) which agrees with (23,36). Moreover, the mean WBC counts was significantly lower in HIE neonates (15.1×103/mm3) in comparison to the control group (17.9×103/mm3). This comes in the line with Engle and Rosenfeld (37) while disagrees with Albanna (23) who didn't find any significant difference of WBCS count between HIE cases and controls. All these hematological findings could be attributed to the effect of hypoxia on bone marrow and pathophysiology of the disease.

Finally, the ROC curve showed high accuracy regarding the use of DDK-1 in detecting mortality and morbidity in hypoxic children which urges its importance as a prognostic tool in HIE neonates.

Conclusion:

The strong correlation detected between high serum Dickkopf-1 and HIE suggest the use of Dickkopf-1 as a predictor for HIE grading. As well, cranial ultrasound simultaneously with serum Dickkopf-1 can be used for the prognosis of HIE.

Limitations:

This study couldn't compare between sonographic changes, different degrees of encephalopathy and serum DKK-1 because severe cases had been died before discharge**.**

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors want to declare no conflict of interests.

Availability of Data:

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request**.**

Disclosure statement:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Authors want to declare no conflict of interests.

List of abbreviations:

AED: Antiepileptic Drug
CBC: Complete blood count
CS: Cesarean section
CVD: Cerebral Vascular Dynamics

DKK-1: Dickkopf-1
EEG: Electroencephalographic
HB: Hemoglobin
HIE: Hypoxic-Ischemic Encephalopathy
HII: Hypoxic-Ischemic Injury
MRI: Magnetic Reasoning Image
PVL: Periventricular Leukomalacia
RI: Resistive Index
US: Ultrasound
WBCs: White Blood Cells

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