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Circulating Dickkopf-1 as a Biomarker in Neonates with Hypoxic Ischemic Encephalopathy (HIE)

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ABSTRACT

Background: The brain damage caused by perinatal hypoxic-ischemic encephalopathy (HIE) can have fatal implications for the baby. Wingless-related integration site (Wnt) signalling is crucial for embryonic cell proliferation, fate determination, and patterning, as Dickkopf-1 (DKK-1) is a secreted protein that plays a role in these processes.

Objective: To understand the role of Dkk-1 in the prognosis of newborns with HIE.

Patients and Methods: From November 2019 to April 2020, at the neonatal intensive care unit (NICU), Menoufia University Hospitals we undertook a case–control research. Two groups of patients: Asphyxiated 15 newborns were included in patient group (Group 1). Group 2: 15 newborns in good health. Blood count, liver and kidney function test, and blood gas analysis were performed. CT or MRI scans were performed. Serum DDK-1 levels at admission and discharge were measured. **Results:** In newborns with HIE, the DKK1 level was greater than in normal neonates. DKK-1 level correlated positively with degree of HIE. DKK-1 level correlated positively with worse outcome among HIE neonates. Serum - level >27 µg /L with a sensitivity of 100% and a specificity of 100% for prediction of mortality in the studied HIE neonates.

Conclusion: There were association between serum DKK-1 level as diagnostic factor and if an Apgar score was less than 3 at the 5th minute, pH less than 7.0, or base excess (BE) less than 12 in the venous or cord blood of neonates within 60 minutes of delivery, asphyxia was diagnosed as a prognostic factor in neonates having HIE.

Keywords: Dickkopfs, Hypoxic-ischemic encephalopathy, Neonates.

INTRODUCTION

The condition known as hypoxic-ischemic encephalopathy affects newborns who have had an acute peripartum or intrapartum incident that results in systemic hypoxemia and/or diminished cerebral blood flow. Having this illness can be fatal and lead to long-term health issues. Asphyxia of the perinatal, delivery, and/or neonatal period can result in the development of HIE **(1).** 23% of all newborn deaths are the result of asphyxia during birth. According to the World Health Organization (WHO), it is one of the highest 20 most common causes of disability in all age groups (in terms of disability-adjusted years of life) (8 percent) **(2).**

To rule out neonatal encephalopathy in infants who are born with low blood oxygen levels, look for symptoms such as seizures or difficulty initiating or maintaining respiration in addition to other signs of neurological dysfunction **(3).**

However, even though hypothermia is the only currently approved treatment for moderate to severe neonatal hypoxic-ischemic encephalopathy, over 45 percent of neonates still have abnormal outcomes despite receiving treatment **(4).**

Biomarkers that can quantify the severity of injury, aid in therapy selection, and provide predictive information are lacking in the management of newborns with HIE. It is being used to diagnose and predict the prognosis of neonatal hypoxic-ischemic encephalopathy **(5).** Hypoxic injury biomarkers have often been conducted several days after birth due to a lack of early sensitivity, and MRI demands that the newborn be moved from the neonatal intensive care unit (NICU) to the imaging room, a job that can be challenging under certain conditions. Head ultrasound (HUS) can be used as an imaging biomarker to overcome both of these issues **(6)**. Because of its lesser sensitivity, HUS has not been thoroughly tested or widely recognized for this purpose. Umbilical arterial blood gases have traditionally been used to evaluate the severity of birth asphyxia, although this technique is ineffective **(7).**

Numerous diseases and disorders are communicated through the Wingless-related integration site (Wnt) family of glycoproteins. Through alterations in proliferation, and survival cells respond to Wnt in a situation-dependent manner. Wnt pathways have been linked to a widevariety of illnesses. It is possible to use Wnt signalling suppression to treat some disorders. The activation of Wnt signalling offers novel therapeutic options for a variety of medical disorders **(8).**

In order to influence cell fate, cell migration, and tissue polarity, Wnt signals are transmitted via the canonical as well as the non-canonical pathways **(9)**. Wnt pathways are regulated by several secreted antagonists, including soluble receptors and Dickkopfs (DKK). Dickkof-1, the most studied of them, inhibits Wnt signalling by binding to LRP5/6 and a cell surface co-receptor, Kremen-1, and promotes receptor complex internalization **(10).**

Serum levels of Dkk-1 are higher in people with stable and unstable angina pectoris than in healthy controls, and this protein has been related to platelet-

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mediated activation of endothelial cells in people with coronary artery disease [11].

In hypoxic settings, Wnt signalling promotes matrix-metalloproteinase-9, which stimulates proliferation and migration of embryonic neural stem cells in culture [12,13]. Dkk-1 may be released into the peripheral circulation during acute ischemia of the brain based on these findings.

It is hoped that this study will help researchers better understand the role of Dkk-1 in the prognosis of newborns with hypoxic ischemic encephalopathy.

SUBJECTS AND METHODS

Subjects:

From November 2019 to April 2020, at the NICU Menoufia University Hospitals we undertook a case–control research.

Ethical consent:

When all participant parents have signed and returned informed consent papers to the Faculty of Medicine, at Menoufia University, the study was approved by the Research Ethics Committee (IRB#6237). For this study, we abided by the principles outlined in the Helsinki Declaration, an ethical framework established by the World Medical Association.

Inclusion criteria:

Two groups of participants were categorized:

15 asphyxiated newborns who met three or more of the clinical and biochemical criteria were included in patient group (Group 1). Group 2: 15 newborns in good health, their pH more than 7.2, Apgar scores at 1 and 5 min more than 7, and no signs of fetal distress, Newborns were considered to have asphyxia if they had a 5-minute Apgar score of less than 3, if their cord blood or venous blood had a pH of less than 7.0, or if their base excess (BE) was less than -12 within the first 60 minutes of life (more than 3 min). A comprehensive medical history review and physical examination was performed on every baby [14].

Exclusion criteria:

Patients with congenital or perinatal infections such as chorioamnionitis and patients with systemic infection such as strep throat have been excluded from the trial. Other exclusion criteria include having a family history of neurological disease, having a consanguineous marriage, or having a history of intrauterine growth retardation.

This is what all of the participants in this research had to go through:

I. **Full history taking stressing on:** (1) Prenatal, natal, postnatal, past family history. (2) The Apgar score of neonates was assessed at 1, 5, and 10 minutes after birth for any history of perinatal asphyxia as well as delivery and maternal history.

II. **Complete physical examination including:**

a. ***General examination:*** (1) Using the new Ballard score for gestational age estimation [15], a person's head circumference and body weight. (2) Vital parameters: assessment of temperature, heart rate, respiratory rate.

b. ***Systemic Examination:*** (1) Within the first 24 hours after birth and for the first seven days after birth, a chest, cardiac, and neurological examination was required to determine if a baby had HIE according to Sarnat Criteria[16]. (2) Neonatology specialists were trained to evaluate a baby's mental and physical well-being, as well as the quantity of anti-convulsant medications he or she has been given.

III. **Laboratory Investigations:**

(a) Routine investigations: (1) Complete blood count: using automated cell counter (coulter) Mindray –BC 3600, China. (2) Liver function test (SGOT, SGPT): using Mindray – BS 800 M., China. (3) Kidney function test (Urea, Creatinine): using Mindray – BS 800 M., China. (4) Blood gases: Method: using Rapid point 500 –Simens, Gu1680D, UK. Value: Umbilical artery blood has a pH of 7.0 and a BE of lower than -12 [17]. (5) Electrolyte's analysis and (6) Random blood sugar.

(b) Specific investigation: DDK-1 serum level at admission and discharge.

Imaging Studies: Head ultrasound in follow up and CT or MRI if needed.

Statistical analysis:

In order to analyze the data acquired, it was loaded into a computer and run via the Statistical Package for the Social Sciences, version 25 (SPSS). Tables and graphs were used to present the findings. The Shapiro–Wilk test was used to examine the distribution properties of variables as well as the homogeneity of variance. The quantitative data was reported in the form of the mean, median, standard deviation, and range. The frequency and proportions of qualitative data were used to present the information. For quantitative independent data, the student's t test (T) and the Mann-Whitney test (MW) were employed to examine the data as needed. To examine qualitatively independent data, researchers employed the Pearson Chi-Square test (χ^2) and Fisher's exact test as needed. P value equals or less than 0.05 was considered significant.

RESULTS

In terms of demographics, there was no statistically significant difference between cases and the control group at 1 minute, 5 minutes, and 10 minutes. There was a statistically significant drop in Apgar score, heart rate (HR), and temperature and statistically significant increase in respiratory rate (RR) in cases compared to controls **(Table 1).**

Table (1): Demographic data, clinical data of studied groups

	Case No=15		Control No=15		P value
	No	%	No	%	
Sex					
Male	7	46.7	8	53.3	0.715
Female	8	53.3	7	46.7	NS
Mode of delivery					
VD	7	46.7	5	33.3	0.456
CS	8	53.3	10	66.7	NS
Gestational age (weeks)					
Mean ± SD	36.40±1.88		37.33±0.81		0.094 NS
Range	33-38		36-38		
Median	37		38		
Weight (kg)					
Mean ± SD	2.79±0.71		3.15±0.48		0.127 NS
Range	1.96-4.32		2.60-3.95		
Median	2.60		2.95		
Maternal age (years)					
Mean ± SD	31.60±5.24		28.40±3.04		0.053 NS
Range	25-40		23-32		
Median	30		29		
Apgar score at 1 minute					
Mean ± SD	1.67±0.816		7.33±0.724		<0.001 HS
Range	1-6		6-8		
Median	2		7		
Apgar score at 5 minutes					
Mean ± SD	3.33±2.09		8.60±0.507		<0.001 HS
Range	1-6		8-9		
Median	2		9		
Apgar score at 10 minutes					
Mean ± SD	5.73±2.01		9.73±0.457		<0.001 HS
Range	3-8		9-10		
Median	5		10		
HC (cm)					
Mean ± SD	37.8±2.13		36.8±1.64		0.609 NS
Range	32-39		35-40		
Median	36		37		
Heart rate (beat/minute)					
Mean ± SD	101.7±30.7		122.3±7.98		0.023 S
Range	55-148		110-135		
Median	102		120		
Respiratory rate (RR) (Breath/minute)					
Mean ± SD	64.9±7.28		49.9±5.88		<0.001 HS
Range	55-75		42-58		
Median	65		50		
Temperature (°C)					
Mean ± SD	36.3±0.516		36.8±0.488		0.017 S
Range	35.5-37		36-37.5		
Median	36		37		

VD= vaginal delivery**, CS=** cesarean section**, NS=** non-significant, **S=** significant**, HS=**highly significant

Consciousness, Moro reflex, suckling reflex, and tone were statistically significantly different between cases and controls. There was statistically significant increase in cases compared to control as regard pH, serum sodium (Na), and serum calcium (Ca) **(Table 2).**

Table (2): Clinical examination and laboratory investigation of studied groups

	Case		Control		
	No=15		No=15		P value
	No	%	No	%	
Consciousness					
Lethargy	9	64.3	0	0	
Hyper alert	3	21.4	15	100	<0.001
Coma	2	14.3	0	0	HS
Moro reflex					
Absent	2	13.3	0	0	
Weak	7	46.7	0	0	0.001
Positive	6	40	15	100	S
Suckling reflex					
Absent Weak	2	13.3	0	0	0.001
Positive	7	46.7	0	0	S
Grasp reflex					
Positive	13	86.7	15	100	0.483
Negative	2	13.3	0	0	NS
Tone					
Normal	2	14.3	15	100	
Flaccid	2	14.3	0	0	<0.001
Hypotonic	10	71.4	0	0	HS
PPHTN					
Normal	13	86.7	15	100	0.483
PHTN	2	13.3	0	0	NS
HIE grad					
Grad 2	12	80	---	----	----
Grad 3	3	20			
Hb (g/dL)					
Mean ± SD	17.04±2.41		17.30±1.79		0.740 NS
WBCs (10^3/uL)					
Mean ± SD	15.55±3.58		12.26±1.86		0.441 NS
Platelets (10^3/uL)					
Mean ± SD	226.6±21.27		199.26±6.7		0.053 NS
pH					
Mean ± SD	7.001±0.128		7.38±0.024		<0.001 HS
Na (mmol/l)					
Mean ± SD	131.86±4.91		137.60±2.29		0.001 S
Ca (mmol/L)					
Mean ± SD	1.04±0.220		1.32±0.239		0.003 S
K ((mmol/L)					
Mean ± SD	4.12±0.63		4.07±0.582		0.819 NS

VD= vaginal delivery**, CS=** cesarean section**, HS=**highly significant**, S=** significant**, NS=** non-significant

There was statistically significant increase in case group compared to controls as regard DKK-1 at admission. There was statistically significant increase as regard DKK-1 at admission compared to its value on discharge in case group. There was statistically significant decrease in survived cases compared to died cases as regard DKK-1 at admission. DKK-1 value at admission was higher than the DKK-1 value at discharge in both survived and died patients; this difference was statically significant. The DKK-1 value at admission was higher than the DKK-1 value at discharge in both grade 2 and grade 3 HIE; this difference was statically significant **(Table 3).**

Table (3): DKK-1 of studied groups at admission and on discharge, and at admissionand on discharge as regard outcome

	At admission (No=15)	On discharge (No=15)	P value
DKK-1 (µg /L)			<0.001
Mean ± SD	23.86±4.20	10.86±3.13	HS
	Survived (No=11)	**Died (No=4)**	**P value**
DKK-1(µg /L)			<0.001
Mean ± SD	22±3.16	29±1.15	HS
Survived =11			
DKK-1(µg /L)			<0.001
Mean ± SD	22±3.16	11±2.93	HS
Died =4			
DKK-1(µg /L)			0.001
Mean ± SD	29±1.15	10.50±4.12	S

HS=highly significant**, S=** significant

There was significant difference in DKK-1 value as regard HIE grades, it was higher in grade 3 than grade 2 **(Table 4)**

Table (4): DKK-1 of studied Patients as regard HIE grades

	Grade 2 (No=12)	Grade 3 (No=3)	P value
DKK-1(µg /L)			
Mean ± SD	22.66±3.79	28.66±1.15	0.02 S

S= significant

There was positive significant correlation between DKK-1 and maternal age, RR, RBCs and creatinine. There was negative significant correlation between DKK-1 and Apgar score at 1, 5 and 10 minutes, temperature, pH, Na and Ca **(Table 5).**

Table (5): Correlation between DKK-1 and other parameters

Parameters	DKK-1	
	r	P value
Gestational age (weeks)	-0.339	0.029 **NS**
Weight (kg)	-0.322	0.083 **NS**
Maternal age (years)	0.323	**0.081 NS**
Apgar score at 1 minute	-0.973	**<0.001 HS**
Apgar score at 5 minutes	-0.962	**<0.001 HS**
Apgar score at 10 minutes	-0.929	**<0.001 HS**
HR (beat / minute)	-0.310	0.096 **NS**
Respiratory rate (Breath/min)	0.753	**<0.001 HS**
Temperature (C°)	-0.441	**0.015 S**
HC (cm)	0.084	0.660 **NS**
Hb (g/dL)	-0.213	0.259 **NS**
RBC (mc/L)	0.351	**0.057 NS**
Platelets (mc/L)	0.288	0.122 **NS**
pH	-0.883	**<0.001 HS**
Na (mmol/L)	-0.562	**0.001 HS**
Ca(mmol/L)	-0.635	**<0.001 HS**
K(mmol/L)	0.142	0.454 **NS**
SGOT (mg/dl)	0.264	0.158 **NS**
SGPT (mg/dl)	0.047	0.804 **NS**
Urea (mg/dl)	0.345	0.062 **NS**
Creatinine (mg/dl)	0.374	**0.042 S**

NS= non-significant, **S=** significant**, HS=**highly significant.

For HIE prediction, DKK-1 had an AUC of 1.00 at cutoff 6.75 **(Table 6, Figure 1).**

Diagnostic performance in predicting severity of HIE was excellent with AUC (0.931) at cutoffs less than 25.50, with 100% sensitivity and 75% specificity, as well as positive and negative predictive values of 79% and 100% respectively **(Figure 2)**. DKK-1 had good prognostic performance in prediction of HIE mortality with AUC (1.00). Cutoff lower than27 had 100% sensitivity and specificity as well as 100% positive predictive value as well as 100% negative predictive value **(Figure 3).**

Table (6): Validity of DKK-1 as a diagnostic marker for HIE

AUC	Significant	Cutoff	Sensitivity	Specificity	NPP	PPV	Accuracy
1.00	0.001	>6.75	100%	86.7%	100%	88%	93%

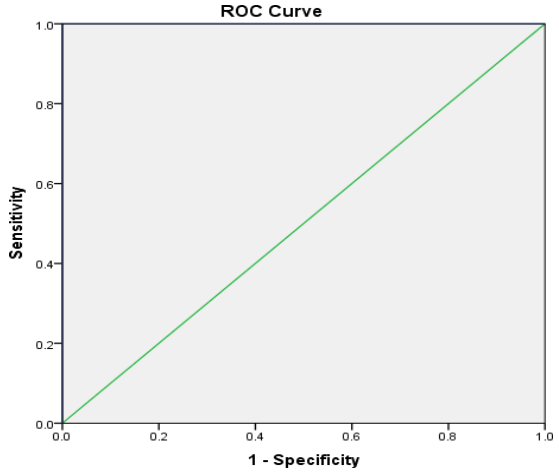

Figure (1): ROC curve of DKK-1 as a diagnostic marker for HIE

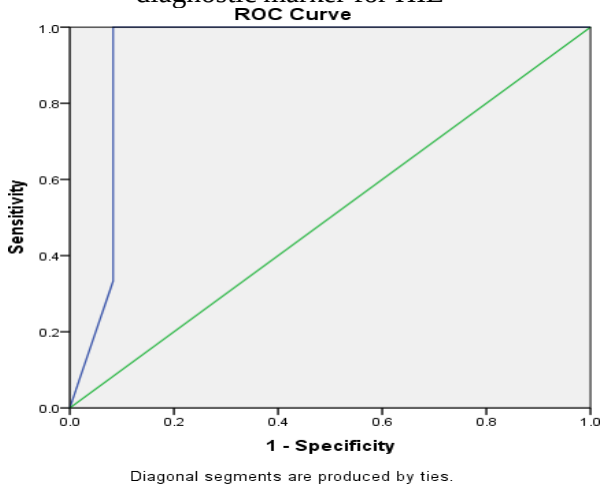

Figure (2): ROC curve of DKK-1 as apredictor marker for HIE severity

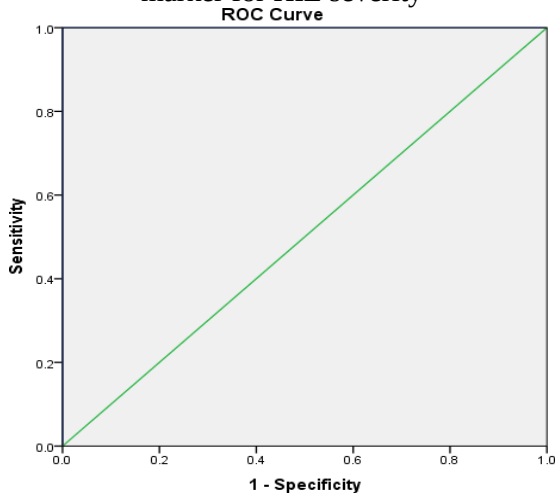

Figure (3): ROC curve of DKK-1 as a predictor marker for HIE mortality

DISCUSSION

In addition to being the biggest cause of newborn death and the primary cause of seizures in term infants, HIE has devastating consequences on the brain's development [18].

In embryogenesis, the Wnt signalling system, which regulates cell patterning, proliferation,and fate determination, is inhibited by DKK1, a secreted protein that plays an important role in the development of the embryo [19]. In order to influence cell fate, cell migration, and tissue polarity, Wnt signals are transmitted via the canonical as well as the non-canonical pathways. Wnt pathways are regulated by a variety of secreted antagonists, including soluble receptors and DKKs. For example, DKK-1, the most extensively researched of these, is found to decrease Wnt signalling by binding to LRP5/6 and a cell surface co-receptor, Kremen-1, which promotes the receptor complex's internalization [20]. Only a few research have examined DKK1 levels as a marker for hypoxic-ischemic encephalopathy (HIE) and its level in various HIE clinical phases caused by neonatal asphyxiation [11].

In our research, there was no discernible difference between the HIE neonates and controls in terms of gestational age, neonatal age, birth weight, gender, or delivery method. In spite of this, there was a substantial disparity in the maternal age of the mothers.

According to **Futrakul *et al.* [21],** infant gestational age did not appear to be a major risk factor in this investigation; nevertheless, the number of male babies among the cases was much higher than in our analysis odds ratio = 2.3 (OR 2.3).

Maternal age was found to be a significant risk factor for HIE in this investigation. Also, in agreement with us, **Varghese *et al.* [22]** study, which found that maternal age >35 years was associated with a higher probability of newborn encephalopathy (OR 4.35). In contrastto us, **Butt *et al.* [23] and Torbenson *et al.* [24];** it was stated that the age of the mother at the time of delivery was insignificant.

Apgar scores were considerably lower in all HIE newborns as compared to controls, and in each HIE neonates group compared to the matching control group in our investigation, as was the case in previous studies [25, 26].

Newborns in the HIE group had significantly lower Apgar scores 7 at 1 and 5 minutes (P0.0001 and 0.0004, respectively) in other studies done by **Jones *et al.* [27] and Thigha *et al.* [28],** although there was no significant difference between the two groups for Apgar score at 10 minutes.

Only three of the 12 newborns with HIE Grade 3 had a statistically significant difference between groups in terms of their grasp Reflex or PPHTN compared to the 12 neonates with HIE grade 2 who had a statistically significant difference in terms of their Moro reflex or suckling reflex.

Most of the patients exhibited mild hypoxic ischemic encephalopathy (43.5%) followed by

moderate and severe hypoxic ischemic encephalopathy (24.8%), according to **Namusoke *et al.*[29]**. 66% perished and 156% were discharged within a week, according to the study. As of the seventh day, the most prevalent problems were the absence of a suckling reaction, a weaker Moro reflex, and the need for nasal prong oxygen therapy.

There was a statistically significant rise in cases compared to control when it came to pH, serum Na, and Ca, but there was no statistically significant difference between groups when it came to HB, WBCs, platelets, and serum K in the current study. For example, according to the findings of **El-Gamasy and Alarabawy [30],** there was an increased incidence of cases, although there was no statistically significant difference between groups in terms of HB, WBCs, platelets and serum K.

Each HIE neonates' group had a lower pH than the respective control group. Hypoxic ischemic newborns were shown to have a higher incidence of anaerobic glycolysis and an increased production of lactic acid (Lactic acidosis) owing to hypoxia, as reported by the **AmericanAcademy of Pediatrics [31] and Karlsson *et al.* [25].** When it comes to platelet counts, patients with HIE were substantially more likely to have low numbers.

In our study, when compared to the control group, there was a statistically significant increase in cases versus controls (23.86 µg /L ±4.2 vs 5.63 µg /L± 0.972) as regard DKK-1 at admission,in addition, there was statistically significant increase as regard DKK-1 at admission compared to its value on discharge (23.86 µg /L ±4.20 vs 10.86 µg /L ±3.13).

According to **Albanna and Ahmed's study[11]**, which looked at the levels of Dickkopf- 1 in hypoxic ischemic newborns, DKK1 was found to be a novel diagnostic marker for HIE in addition to clinical diagnosis as DKK1 increase in HIE group than control group and its level was higher in severe HIE neonates as compared to moderate and mild HIE neonates, so they suggested that DKK1 is useful prognostic factor. Also, HIE newborns had significantly greater serum DKK1 levels than controls, according to **Afifi *et al.* [32]** (10.5±5.43 vs 1.1±0.65 respectively).

We studied the comparison of serum DKK1 level between HIE neonates with different degrees of hypoxia and we found that serum levels of DKK1 was significantly higher in grade 3 HIE neonates (28.66±1.15) as compared to grade 2 HIE neonates (22.66±3.79), so DDK1 is useful as a diagnostic marker for HIE. In addition, the DKK-1 value at admission was higher than the DKK-1 value at discharge in both grade 2 and grade 3 HIE. This difference was statically significant.

This is in agreement with **Albanna and Ahmed [11]** study, which found that in patients with severe HIE, plasma levels of DKK-1 are markedly elevated (1193.1 µg /L ± 5.37) versus moderate (992.9 µg /L ± 102.9) as well as mild cases (782.8 µg /L ± 136.2), so DKK-1 can be used to predict the future. Serious HIE patients with sequelae or death have elevated DKK-1 levels in their blood.

In present study, there was positive significant correlation between DKK-1 and maternal age, RR, RBCs and creatinine. There was negative significant correlation between DKK-1 and Apgar score at 1, 5 and 10 minutes, temperature, pH, Na and Ca. No significant correlation between DKK-1 and gestational age, weight, HR, Hb, HC, platelets, K, SGOT, SGPT and urea was found.

In agreement with **Albanna and Ahmed [11]** study, the plasma DKK-1 levels of men and women, as well as those of full-term and preterm inpatients, were not significantly different.

When we plotted the ROC curve for detection of HIE, we found that DKK-1 had excellent diagnostic performance, with 100% sensitivity, 86.7 % specificity, positive predictive value (88 %), and negative predictive value (100 %). As prediction of HIE severity at cutoff >25.50 µg /L it gave (100%) sensitivity, specificity (75%), positive predictive value (79%) and negative predictive value (100%) while at cutoff >27 µg /L give sensitivity (100%), specificity (100%) in prediction of HIE mortality.

Albanna and Ahmed [11] study reported that, cutoff value of plasma DDK-1 level was 470 pg/ml and 95% accuracy 95.0 sensitivity, 90.0 specificity, 90.5 PPV and AUC 0.967 (0.904–1.03). **Afifi *et al.* [32],** Showed that using the ROC curve, serum DKK1level >11µg/L in neonates with HIE could significantly predict mortality of these studied HIE neonates with a sensitivity of 85.7% and a specificity of 76.9%.

CONCLUSION

Our study found that, there was association between serum DKK-1 level as diagnostic and prognostic factor in neonates with HIE.

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