

*Research Article***Serum Dickkopf-1 level in neonates with Hypoxic-Ischemic Encephalopathy****Mohamed F. Afifi***, **Laila E. Aboul-Fotoh***, **Mostafa A. El-Sayed Ahmed****, and **Mona M. Hassan*****

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Abstract**Objective:** To measure the serum Dickkopf-1 level in neonates with HIE and to study its relation to the neurodevelopmental outcome in those neonates in comparison to healthy neonates.**Methods:** We measured serum levels of Dkk-1 by ELISA in neonates with HIE (n = 40) and in healthy controls (n = 30). **Results:** Dkk-1 serum levels increased significantly in HIE neonates than in healthy control, Serum DKK-1 levels increased significantly in severe HIE patients. **Conclusion:** DKK1 level was higher in neonates with HIE than normal neonates and DKK1 level correlated positively with degree of HIE.**Keywords:** Dickkopf-1, neonates, Hypoxic-Ischemic**Introduction**

Neonatal hypoxic-ischemic insults are a significant cause of pediatric encephalopathy, developmental delays, and spastic cerebral palsy. Although the developing brain's plasticity allows for remarkable self-repair, severe disruption of normal myelination and cortical development up on neonatal brain injury are likely to generate life persisting sensory-motor and cognitive deficits in the growing child (Chicha et al., 2014).

HIE has an incidence of 3 to 5 per 1000 live births in the developed world and remains associated with significant mortality and neurodevelopment sequel (Natarajan et al., 2014).

The incidence in developing countries range from 2.3 – 26.5 per 1,000 live births (Horn et al., 2013).

Human Dickkopf-1 (DKK1) is a member of the Dickkopf gene family, which is composed of Dickkopf-1, Dickkopf-2, Dickkopf-3, and Dickkopf-4, together with a unique Dickkopf-3-related protein termed Soggy (Katoh and Katoh, 2005).

Among the members of the Dickkopf family, Dickkopf-1 is a secreted protein involved in

embryonic development and known as a potent inhibitor of the Wnt signaling pathway, which plays a critical role in cell patterning, proliferation, and fate determination during embryogenesis (Ogoshi et al., 2011).

In the embryonic brain, Wnt signaling induces self-renewal of radial glia progenitors and differentiation, but not proliferation, of intermediate progenitors (Munji et al., 2011).

Forced expression of Dkk1 severely reduces neurogenesis in the developing hippocampus (Solberg et al., 2008).

Recent findings have pointed to an increased expression of DKK1 causally related to neurodegenerative processes associated with Alzheimer's disease or brain ischemia (Rosi et al., 2010).

Subjects and Methods

This comparative study included 70 neonates who were collected from the neonatal intensive care unit and the outpatient neonatal clinic, Maternity & Children's Minia University Hospital during the period from March 2016 to December 2016. Laboratory investigations were performed at Clinical Pathology department-

Minia University Hospital. Studied neonates were divided into four groups:-

Group I: (Study group) (full term HIE neonates) included 20 full term (≥ 37 weeks) neonates with hypoxic ischemic encephalopathy (10 males, 10 females), their ages ranged from 1-9 days.

Group II: (Study group) (preterm HIE neonates) included 20 preterm (< 37 weeks) neonates with hypoxic ischemic encephalopathy (13 males, 7 females), their ages ranged from 1-12 days.

Group III: (Control group) (full term controls) included 15 apparently healthy full term (≥ 37 weeks) neonates (12 males, 3 females), their ages ranged from 1-8 days.

Group IV: (Control group) (preterm controls) included 15 apparently healthy preterm (< 37 weeks) neonates (11 males, 4 females), their ages ranged from 1-8 days.

❖ **Inclusion criteria:**

Hypoxic full term and preterm neonates with age < 2 weeks were diagnosed according to Sarnat criteria and were divided into 2 groups:

- 20 full term neonates (10 males, 10 females), their gestational ages ranged from 37-42 weeks, their ages ranged from 1-9 days.
- 20 preterm neonates (13 males, 7 females), their gestational ages ranged from 31-36 weeks, their ages ranged from 1-12 days.

❖ **Exclusion Criteria:**

1. Maternal diseases as (hypertension, diabetes mellitus, anemia, cardiac and neurological diseases).
2. Associated congenital anomalies.
3. Associated diseases as (sepsis, respiratory distress, jaundice and CNS infection).

❖ **Diagnosis of HIE in our neonates:**

All studied neonates were subjected to the following:

1- Complete medical history....(Prenatal, natal and post natal history of diseases and medications).

2- Thorough Clinical examination

With stress on signs of encephalopathy in the form of

a) According to Sarnat classification of HIE

1- Disturbed conscious level.

2- Abnormal neuromuscular control in the form of (abnormal muscle tone, abnormal posture, abnormal stretch reflexes and presence or absence of segmental myoclonus).

3- Abnormalities in complex reflexes in the form of (suckling, moro, oculo-ves-tibular and tonic neck).

4- Abnormal autonomic function.

5- Abnormal pupil size.

6- Abnormal respiration.

7- Abnormal heart rate.

8- Abnormal GIT motility.

9- Presence or absence of seizures (Sarnat and Sarnat, 1976).

b) Apgar score < 6 at 5 minutes (ACOG., 2014).

3- Laboratory investigations including the following:

- Arterial cord blood gases: **PH < 7.0 and Base excess (BE) < -12 in umbilical artery blood (White et al., 2010).**

The kit is for the quantitative level of DKK1 in the sample, use purified Human DKK1 antibody to coat microtiter plate wells, make solid-phase antibody, then add DKK1 to wells. Combined DKK1 antibody which with enzyme labelled, become antibody-antigen - enzyme-antibody complex, after washing completely, add substrate, substrate becomes blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of DKK1 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Statistical analysis

Data entry and analysis were all done with IBM compatible computer using software "SPSS for windows version 22". Graphics were done by Excel Microsoft office 2010.

Quantitative data were presented by mean and standard deviation, while qualitative data were presented by frequency distribution. Chi square test was used to compare between proportions. Student t-test was used to compare between two groups.

Pearson correlation test used to associate between two quantitative variables, and Spearman correlation to associate between one quantitative and other ordinal variable.

The accuracy of DDK-1 for detecting morbidity and mortality among hypoxic children was assessed using ROC curve, which plot the

sensitivity (true-positive rate) to the false-positive rate (1 – specificity). A statistically significant level was considered when p value was less than 0.05.

Results

Table (1): Comparison between HIE neonates and controls regarding serum DKK1 level

| Serum DKK1 (µg/l) | Patients N=40 | Controls N=30 | P-value |
|-------------------|------------------|------------------|----------|
| Range | 2.3-19.7 | 0.1-2.2 | 0.0001** |
| Mean±SD | 10.5±5.43 | 1.1±0.65 | |

*= Significant, **= highly significant

Table (1) showed that serum DKK1 level was statistically significant higher in HIE neonates than controls.

Table (2): Cutoff value, sensitivity and specificity of serum DKK1 level for prediction of mortality among HIE neonates

| | Cut off | Sensitivity | Specificity | Positive PV | Negative PV |
|------------------|---------|-------------|-------------|-------------|-------------|
| Serum DKK1 µg /l | >11 | 85.7% | 76.9% | 66.7% | 90.9% |

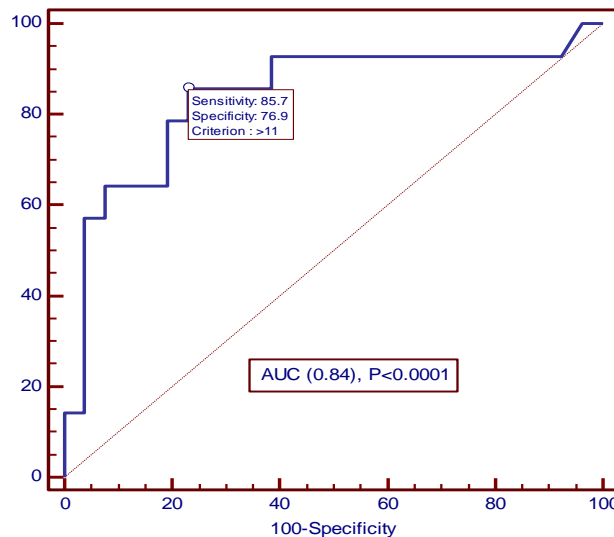


Figure (1): Receiver operating characteristic curve (ROC) curve of serum DKK1 level for prediction of mortality among HIE neonates

Table (2) showed that using the ROC curve, serum DKK₁ level >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% as shown in figure (1).

Conclusions

- DKK1 is a good diagnostic and prognostic factor for hypoxic ischemic encephalopathy.
- DKK1 level was higher in neonates with HIE than normal neonates.
- DKK1 level correlated positively with degree of HIE.

References

- 1- Chicha L, Smith T and Guzman R. Stem cells for brain repair in neonatal hypoxia-ischemia. *Childs Nerv Syst*, 2014; 30: 37-46.
- 2- Natarajan G, Shankaran S, Pappas A, Bann C, Tyson JE, McDonald S, Das A, Hintz S, Vohr B and Higgins R. Functional status at 18 months of age as a predictor of childhood disability after neonatal hypoxic-ischemic encephalopathy. *Dev Med & Child Neurol*, 2014; 56: 1052-1058.
- 3- Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, Tooke L, Rhoda NR and Robertson NJ. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. *J Perinat Med*, 2013;41(2):211–217.
- 4- Katoh K and Katoh M. Comparative genomics on DKK2 and DKK4 orthologs. *International Journal of Molecular Medicine*, 2005; 16: 477–481.
- 5- Ogoshi K, Kasamatsu A, Iyoda M, Sakuma K, Yamatoji M, Sakamoto Y, Ogawara K, Shiiba M, Tanzawa H and Uzawa K. Dickkopf-1 in human oral cancer. *International Journal of Oncology*, 2011; 39: 329–336.
- 6- Munji RN, Choe Y, Li G, Siegenthaler JA and Pleasure SJ. Wnt signaling regulates neuronal differentiation of cortical intermediate progenitors. *J Neurosci*, 2011; 31: 1676–1687.
- 7- Solberg N, Machon O and Krauss S. Effect of canonical Wnt inhibition in the neurogenic cortex, hippocampus, and premigratory dentate gyrus progenitor pool. *Dev Dyn*, 2008; 237: 1799–1811.
- 8- Rosi MC, Luccarini I, Grossi C, Fiorentini A, Spillantini MG, Prisco A, Scali C, Gianfriddo M, Caricasole A, Terstappen GC and Casamenti F. Increased Dickkopf-1 expression in transgenic mouse models of neurodegenerative disease. *J Neurochem*, 2010; 112:1539–1551.
- 9- Sarnat HB and Sarnat MS. Neonatal encephalopathy following fetal distress: A clinical and electroencephalographic study. *Arch Neurol*, 1976; 33: 695 - 706.
- 10- American College of Obstetrics and Gynecology. Task Force on Neonatal Encephalopathy; American Academy of Pediatrics. Neonatal Encephalopathy and Neurologic Outcome, 2nd edition. Washington, DC: American College of Obstetricians and Gynecologists. 2014.
- 11- White CR, Doherty DA, Henderson JJ, Kohan R, Newnham JP and Pennell CE. Benefits of introducing universal umbilical cord blood gas and lactate analysis into an obstetric unit. *Australia and New Zealand J of Obstetrics and Gynaecology*, 2010; 50: 318-328.