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RESEARCH ARTICLE

# Vitamin D as an Adjuvant Therapy in Neonatal Hypoxia: Is it Beneficial?

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**Abstract:** ***Background:*** Neonatal hypoxic ischemic encephalopathy (HIE) is a potentially devastating disorder associated with significant mortality and long-term morbidity.

***Objective:*** The aim of this study was to study the role of vitamin D as an adjuvant therapy for management of neonatal HIE.

***Patients and Methods:*** This study was carried out on 60 neonates with HIE grade II who were diagnosed according to modified Sarnat staging and were divided in to 2 groups: **Group I:** Included 30 neonates with Sarnat grade II HIE who received single daily oral dose of vitamin D3 (1000 IU) for 2 weeks in addition to daily subcutaneous (SC) human recombinant erythropoietin (2500 IU/kg) for 5 days and IM or IV magnesium sulphate 250 mg/kg within half an hour of birth, and subsequently 125 mg/kg at 24 and 48 hours of life. **Group II**: Included 30 neonates with HIE grade II who received erythropoietin and magnesium sulphate as group I but without vitamin D. Two blood samples were taken from all neonates included in both groups; the 1st at diagnosis and the 2nd after 2 weeks of therapy. This study included also 30 healthy neonates as a control group. All neonates included in this study were subjected to: complete clinical examination with assessment of Apgar score at 5 and 10 minutes, measurement of arterial blood gases and serum 25 (OH) vitamin D, calcium, phosphorus, S100-B and IL-17 levels.

***Results:*** Before therapy, there were no significant differences between group I and II in PH, PO2 and PCO2 (p= 0.294, 0.462, 0.758 respectively), but after 2 weeks of therapy, there were significantly higher PH levels in group I compared with group II (p <0.001) while there were no significant differences between group I and II regarding PO2 and PCO2. Before therapy, there were no significant differences in serum 25(OH) vitamin D levels between group I and II while there were significantly lower serum 25(OH) vitamin D levels in group I and II compared with controls (P1; comparison between group I and II = 0.742, P2; comparison between group I and controls = 0.001 and P3; comparison between group II and controls = 0. 001). There were no significant differences between group I and II and between group I and II and control as regard serum calcium (P1= 0.943, P2= 0.875 and P3= 0.764) and phosphorus (P1= 0.862, P2= 0.921, P3= 0.786). There were no significant differences between group I and II regarding serum IL-17 levels while there were significantly lower serum IL-17 levels in group I and II compared with controls (P1 = 0.457, P2 = 0.043 and P3 = 0.023). Before therapy, there were no significant differences in serum S100-B levels between group I and II while there were significantly higher serum S100-B levels in group I and II compared with control (P1 = 0.381, P2 = 0.001 and P3= 0.001) but after therapy, there were significantly higher S100-B levels in group II compared with group I and significantly higher S100-B levels in group I and II compared with control (P1= 0.001, P2= 0.043, P3 = 0.001). There were significant negative correlations in group I between serum S100-B and PH and between S100-B and serum vitamin D before and after therapy.

***Conclusion:*** Vitamin D was found to improve the cases of group I as demonstrated by the reduction of serum S100-B levels after vitamin D therapy.

***Recommendations:*** Extensive multicenter studies are required on a large number of patients with Sarnat grade II HIE with longer duration of follow up to give valid recommendations about the use of vitamin D as an adjuvant therapy in Sarnat grade II HIE.

**Keywords:** Hypoxic ischemic encephalopathy, S100-B, Vitamin D status, neonates, erythropoietin, seizures.

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## 1. INTRODUCTION

Neonatal hypoxic ischemic encephalopathy (HIE) is a serious disease that may lead to permanent brain injury. HIE has serious effects on the developing brain and is considered one of the most common causes of morbidity and mortality among neonates, also it is one of the most important causes of seizures in neonates and cerebral palsy in infants and children [1].

Neonatal HIE is a common problem in developing countries resulting in a high incidence of neonatal morbidity and

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mortality [2]. HIE usually presents clinically in the first 6 hours of the neonatal period with difficulty in initiating and maintaining breathing, hypotonia and hyporeflexia, decreased levels of consciousness and seizures [3]. Neonatal HIE is classified according to Sarnat grading system into mild, moderate, and severe HIE, based on clinical symptoms (like consciousness, tone, reflexes and seizures) and electroencephalogram evaluation [4]. Mild grade of neonatal HIE has no effect on brain function, the moderate grade is associated with a moderate effect on brain function and the severe grade is associated with marked permanent brain damage [3]. The pathogenesis of neonatal HIE consists of primary energy failure phase at the cellular level which occurs in the first 6 hours after hypoxic ischemic episode. Second energy failure phase occurs after 6-48 hours of hypoxic ischemic episode [4].

Adequate management of HIE in neonates by focusing on new strategies gives a better prognosis and decreases the morbidity and mortality from HIE [5]. These measures with neuroprotective effects include head and total body cooling, erythropoietin, melatonin and magnesium sulphate [6].

S-100 proteins are a family of $Ca^{2+}$-binding cytosolic proteins that are expressed in multiple cell types and contain 2 subunits (α and β); these proteins are involved in cell cycle progression, cell development, cytoskeletal-membrane interactions, and cell differentiation [7]. S-100β is expressed in glial cells in the nervous system; many studies have stated that the serum S-100β concentration is an important predictor of severity of Hypoxic-ischemic encephalopathy [8, 9].

Magnesium sulfate ($MgSO_4$) is essential for the key cellular processes, like glycolysis, oxidative phosphorylation, proteins synthesis, and DNA and RNA aggregation. $MgSO_4$ has been found to block NMDA receptors occupying the binding sites within these ion channels preventing the neuronal cell damage caused by activation of these receptors and this could explain the neuro-protective effect of $MgSO_4$ in cases of Hypoxic-ischemic encephalopathy [10].

Erythropoietin (Epo) is an endogenous cytokine that regulates hematopoiesis and is used to treat anemia. In addition, it has recently increased interest in the neurosciences since the new concept of Epo as a neuroprotective agent has emerged [1].

The cellular mechanisms by which EPO exerts neuroprotection are complex and not completely understood. Binding of EPO to its receptor leads to phosphorylation of both EPO-R and the signaling protein Janus kinase 2 (JAK2), which provides a docking complex for intracellular signaling proteins including phosphatidylinositol 3-kinase (PI3K) and Akt, STAT5, and the extracellular signal-regulated kinase ERK. Phosphorylation and activation of these pathways affect a number of downstream targets that alter cell survival, proliferation, and differentiation. Akt limits inflammation and decreases apoptotic cell death, while STAT5 plays a role in cell survival. The ERK pathway has anti-apoptotic and anti-inflammatory effects *in vitro* and is also critical for neurogenesis and cell fate commitment [11].

Vitamin D is a hormone that has many functions within the body. It has been associated with calcium and bone metabolism, but more recently has been demonstrated to be a vital component in neuronal development and function and may provide neuroprotection. Vitamin D is also a potent neurohormone, with vitamin D receptors and several enzymes in the vitamin D synthetic pathway found throughout the brain. The dose and level of vitamin D that would potentially be necessary to provide neuroprotection is unknown. The American Academy of Pediatrics recommendation currently states that all healthy term infants should be supplemented with 400 IU/day although other societies have recommended doses as high as 1000 IU/day [12].

### 1.1. Objective

The aim of this study was to study the role of vitamin D as an adjuvant therapy for the management of neonatal hypoxic-ischemic encephalopathy.

## 2. PATIENTS AND METHODS

This prospective clinical trial was performed at neonatal ICU, Pediatric Department, Tanta University Hospital over the period from January 2017 to March 2018 after approval by the Ethical Committee of Faculty of Medicine, Tanta University and informed consents were taken from the parents of all neonates included in the study. This prospective clinical trial was carried out on 60 neonates with grade II HIE who were diagnosed according to the modified Sarnat staging and were divided into 2 main groups:

**Group I:** Included 30 neonates with HIE Sarnat grade II [13] who received single daily oral dose of vitamin D (1000 IU) for 2 weeks in the form of Vidrop which is stabilized aqueous solution of vitamin D3 (Cholicalciferal) with each 1ml =28 drops containing 2800 units oral solution of Cholicalciferal and each drop contains 100 units of vitamin D3 (Medical union pharmaceutical) [14] in addition to daily SC human recombinant erythropoietin (2500 IU/kg) for 5 days [15] and IM or IV magnesium sulphate 250 mg/kg within half an hour of birth, and subsequently 125 mg/kg at 24 and 48 hours of life [16].

**Group II**: Included 30 neonates with Sarnat grade II HIE [13] who received human recombinant erythropoietin (2500 IU/kg) for 5 days [15] and IM or IV magnesium sulphate 250 mg/kg within half an hour of birth, and subsequently 125 mg/kg at 24 and 48 hours of life [17-19]. Two blood samples were taken from all neonates included in both groups; the $1^{st}$ at diagnosis and the $2^{nd}$ after 2 weeks of therapy.

This study included also 30 healthy neonates as a control group to detect vitamin D and S100 protein levels.

### 2.1. Magnesium Sulphate Dosage and Administration

IM or IV Magnesium sulphate 250 mg/kg was given within half an hour of birth, and subsequently 125 mg/kg at 24 and 48 hours of life [16-18]. For IV administration: Concentration should not exceed 200 mg/mL (20%) [17] and rate of administration usually not exceed 150 mg/minute (e.g., 1.5 mL/minute of 10% concentration or equivalent) [19]. For IM administration: Concentration ≤200 mg/mL was used (20%) [20] and dosages were adjusted carefully according to

individual requirements and response and was discontinued as soon as the desired effect was obtained [19].

Patients of Group I and II who were diagnosed as Sarnat grade II HIE [13] should fulfil two of the following criteria (Inclusion criteria):

1. Apgar score of less than 5 at 5 and 10 minutes.

2. Umbilical artery acidemia (PH less than 7 and/or base deficit≥ 12mmol/L)

3. Evidence of Sarnat grade II HIE using modified Sarnat score Table **1** [13].

**Table 1. Sarnat grading HIE.**

| | Mild HIE (I) | Moderate HIE (II) | Severe HIE (III) |
|---|---|---|---|
| Levels of consciousness | Hyper alert | Lethargic | Stuporose |
| Muscle tone | Normal | Mild hypotonia | Flaccid |
| Suckling reflex | Normal/weak | Weak/absent | Absent |
| Moro reflex | Strong | Weak/incomplete | Absent |
| Seizures | Absent | Common | Frequent/difficult to control |

**Exclusion criteria:** congenital anomalies, intra uterine growth retardation (I.U.G.R), neonatal sepsis, infant of diabetic mother (I.D.M).

**All neonates in the study were subjected to the following:**

Assessment of Apgar score at 5 and 10 minutes.

Complete clinical examination.

Assessment of arterial blood gases (ABG).

Assessment of serum 25 (OH) vitamin D, calcium and phosphorus levels.

Assessment of serum S100-B levels.

Assessment of IL-17 levels.

### 2.2. Blood Sampling

Venous blood samples were aseptically collected in sterile plain tubes, centrifuged at 3000 rpm for 10 min at 4°C to obtain serum which was stored at -20°C till analysis of serum 25(OH) vitamin D by commercial assay kit supplied by Chongqing Biospes Co., Ltd., China.

### 2.3. Principle of 25(OH) Vitamin D Assay

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with 25(OH) vitamin D. During the reaction, 25(OH) vitamin D in the sample or standard competes with a fixed amount of 25(OH) vitamin D on the solid phase supporter for sites on the biotinylated detection antibody specific to 25(OH) vitamin D. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of 25(OH) vitamin D in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### 2.4. Enzyme Linked Immunosorbent Assay (ELISA) for S100-B

Serum S100-B levels were immunoassayed using commercial kits supplied by Chongqing Biospes Co., Ltd., City, China. ELISA technique was done according to the manufacturer's protocol and read on microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France), at 450 nm with correction wavelength set at 570 nm.

### 2.5. Enzyme Linked Immunosorbent Assay (ELISA) for Assessment of IL-17

Serum IL-17 levels were measured by enzyme-linked immunosorbent assay (ELISA) technique (enzyme-amplified sensitivity immunoassay (EASIA) kits, Bio Source Europe SA, 8 B-1400, Nivelles, Belgium). These assays detected only human cytokines and the minimum detectable concentration was 4.6 pg. /mL for IL-17 [21].

### 2.6. Blood Gases Assessment

Umbilical cord or radial or femoral artery blood samples were collected using heparinized disposable syringes (2ml syringe washed by 1000IU/ ml heparin) and the samples were analyzed by Blood Gas Analyser –Bayer, Germany. It directly measures PH, PCO2, bicarbonate, base deficit [22].

### 2.7. Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Sciences, version 14 for windows (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviation. Paired and unpaired t-test for comparison between means of two groups was performed.

## 3. RESULTS

There were no significant differences between patients and controls regarding weight, gestational age, sex and mode of delivery Table **2**.

Before therapy, there were no significant differences in serum 25(OH) vitamin D levels between group I and group II while there were significantly lower serum 25(OH) vitamin D levels in group I and group II compared with controls (P1; comparison between group I and II = 0.742, P2; comparison between group I and controls = 0.001* and P3; comparison between group II and controls = 0. 001*) Table **3**.

There were no significant differences between group I and group II and between group I and group II and controls as regard serum calcium (P1= 0.943, P2 = 0.875, P3 = 0.764) and phosphorus levels (P1= 0.862, P2 = 0.921, P3= 0.786) Table **3**.

There were no significant differences between group I and group II regarding serum IL-17 levels while there were

**Table 2. Comparative characteristics between studied groups.**

| Parameters | | Group I (n=30) | Group II (n=30) | Controls (n=30) | t. test | P. value |
|---|---|---|---|---|---|---|
| Weight (kg) | Mean ± SD | 2657.47± 41.86 | 2648.37± 39.87 | 2677.67± 45.15 | 2.745 | 0.249 |
| Gestational age (weeks) | Mean± SD | 37.93 ± 1.41 | 37.98 ± 1.52 | 38.60 ± 1.24 | 2.189 | 0.178 |
| | | **Number (%)** | **Number (%)** | **Number (%)** | **$X^2$** | **P. value** |
| Mode of delivery | NVD | 16 (53.33%) | 16 (53.33%) | 16 (53.33%) | | |
| | CS | 14 (46.7%) | 14 (46.7%) | 14 (46.7%) | 0.000 | 1.000 |
| Sex | Males | 18 (60%) | 18 (60%) | 16 (53.33%) | | |
| | Females | 12 (40%) | 12 (40%) | 14 (46.7%) | 0.364 | 0.833 |

*P. value is significant if < 0.05. NVD: Normal vaginal delivery. **CS:** Cesarean section.

**Table 3. Comparison between Group I and Group II regarding serum 25 (OH) vitamin D, calcium, phosphorus and IL-17 levels before and after vitamin D administration.**

| Vitamin D Status and IL-17 Before Therapy (ng/ml) | | | | | |
|---|---|---|---|---|---|
| **Parameters** | **Group I (No=30)** | **Group II (No=30)** | **Controls (No=30)** | **t .test** | **P values** |
| **Serum 25 (OH) vitamin D** | | | | t1=8.322 | P1=0.742 |
| **Range** | 14.34-19.60 | 14.30- 19.33 | 20 -32 | t2=157.412 | P2=0.001* |
| **Mean ± SD** | 16.95±2.60 | 17.15±2.88 | 24.36±3.35 | t3=132.411 | P3=0.001* |
| **Serum calcium (mg/dl)** | | | | t1=0.031 | P1=0.943 |
| **Range** | 8.5-10.7 | 8.5-10.8 | 8.5-10.9 | t2=0.231 | P2=0.875 |
| **Mean ± SD** | 10.11±0.43 | 10.09±0.44 | 10±0.42 | t3=0.423 | P3=0.764 |
| **Serum phosphorous (mg/dl)** | | | | t1=0.043 | P1=0.862 |
| **Range** | 2.7-7.7 | 2.8-7.8 | 2.9-7.5 | t2=0.048 | P2=0.921 |
| **Mean ± SD** | 5.20±0.50 | 5.22±0.51 | 5.25±0.53 | t3=0.054 | P3=0.786 |
| **IL-17 (pg/ml)** | | | | t1=2.197 | P1=0.457 |
| **Range** | 14.6-6.24 | 14.8-5.72 | 8.1-10.25 | t2=3.912 | P2=0.043* |
| **Mean ± SD** | 15.2± 1.98 | 15.1± 2.43 | 8.7± 2.56 | t3=3.657 | P3=0.023* |
| **Vitamin D Status and IL-17 After Therapy (ng/ml)** | | | | | |
| **Parameters** | **Group I (No=30)** | **Group II (No=30)** | **Controls (No=30)** | **t .test** | **P values** |
| **Serum 25 (OH) vitamin D** | | | | T1=68.412 | P1=0.001* |
| **Range** | 20 -30 | 14.30- 19.33 | 20 -32 | t2=47.238 | P2=0.867 |
| **Mean ± SD** | 24 ± 3 | 17.15±2.88 | 24.36±3.35 | t3=132.411 | P3=0.001* |
| **Serum calcium (mg/dl)** | | | | t1=0.031 | P1=0.943 |
| **Range** | 8.5-10.7 | 8.5-10.8 | 8.5-10.9 | t2=0.231 | P2=0.875 |
| **Mean ± SD** | 10.11±0.43 | 10.09±0.44 | 10±0.42 | t3=0.423 | P3=0.764 |
| **Serum phosphorous (mg/dl)** | | | | t1=0.043 | P1=0.862 |
| **Range** | 2.7-7.7 | 2.8-7.85. | 2.9-7.5 | t2=0.048 | P2=0.921 |
| **Mean ± SD** | 5.20±0.50 | 5.22±0.51 | 5.25±0.53 | t3=0.054 | P3=0.786 |
| **IL-17** | | | | T1=11.34 | P1=0.001* |
| **Range** | 8.5-10.11 | 4.8-5.72 | 8.1-10.25 | t2=6.754 | P2=0.743 |
| **Mean ± SD** | 8.9±2.31 | 5.1± 2.43 | 8.7± 2.56 | t3=3.657 | P3=0.023* |

* Significant P<0.05. T1and P1=comparison between group I and II. T2 and P2= comparison between group I and controls. T3 and P3= comparison between group II and controls.

significantly lower serum IL-17 levels in group I and group II compared with controls (P1 = 0.457, P2 = 0.043*, P3 = 0.023*) Table **3**.

Before therapy, there were no significant differences in serum S100-B levels between group I and group II while there were significantly higher serum S100-B levels in group

I and group II compared with controls (P1 = 0.381, P2 = 0.001* and P3= 0.001*) but after therapy, there were significantly higher S100-B levels in group II compared with group I and significantly higher S100-B levels in group I and group II compared with control (P1= 0.001*, P2= 0.043*, P3 = 0.001*) Table **4**.

Before therapy, there were no significant differences between group I and group II in PH, PO2 and PCO2 (p= 0.294, 0.462, 0.758 respectively), but after therapy, there was significantly higher PH level in group I compared with group II (p <0.001*) while there were no significant differences between group I and group II regarding PO2 and PCO2 Table **5**.

There were significant negative correlations in group I between serum S100-B and PH before and after therapy and significant negative correlations between serum level of vitamin D and S100-B before and after 2 weeks therapy Table **6**.

**Table 4. Comparison between Group I and Group II according to serum S100-B level (µg/L).**

| S100-B (µg/L) | Group I (n=30) | Group II (n=30) | Controls (n=30) | t .test | P values |
|---|---|---|---|---|---|
| **Before therapy** | | | | | |
| Range | 10-18 | 10-18 | 0.32–1.23 | t1= 12.34 | P1= 0.381 |
| Mean ± SD | 13.60 ± 2.42 | 13.40 ± 2.38 | 0.69 ± 0.27 | t2= 10.874 | P2= 0.001* |
| Median | 13.5 | 13.2 | 0.96 | t3= 9.349 | P3= 0.001* |
| **After therapy** | | | | | |
| Range | 1-7 | 8-14 | 0.32–1.23 | t1= 7.652 | P1 = 0.001* |
| Mean ± SD | 3.50 ± 1.89 | 9.90 ± 2.03 | 0.69 ± 0.27 | t2= 5.651 | P2 = 0.043 |
| Median | 3 | 10.5 | 0.96 | t3= 6.451 | P3 = 0.001* |

* Significant P<0.05. T1and P1=comparison between group I and II. T2 and P2= comparison between group I and controls. T3 and P3= comparison between group II and controls.

**Table 5. Comparison between Group I and Group II according to main elements of blood gases.**

| - | Group I (No=30) | Group II (No=30) | t .test | P values |
|---|---|---|---|---|
| **PH** | | | | |
| **Before vitamin D administration** | | | | |
| **Range** | 7.05-7.15 | 7.04 – 7.15 | | |
| **Mean ± SD** | 7.09 ± 0.03 | 7.08 ± 0.03 | 10.34 | 0.294 |
| **After 2 weeks of vitamin D therapy** | | | | |
| **Range** | 7.33 – 7.43 | 7.14 – 7.29 | | |
| **Mean ± SD** | 7.38 ± 0.03 | 7.19 ± 0.05 | 5.32 | <0.001* |
| **Po2** | | | | |
| **Before vitamin D administration** | | | | |
| **Range** | 77-90 | 76-90 | | |
| **Mean ± SD** | 80.72±4.339 | 80.66±3.541 | 7.66 | 0.462 |
| **After 2 weeks of vitamin D therapy** | | | | |
| **Range** | 80-90 | 80-90 | | |
| **Mean ± SD** | 83.319±3.218 | 82.241±3.631 | 6.247 | 0.458 |
| **Pco2** | | | | |
| **Before vitamin D administration** | | | | |
| **Range** | 35-45 | 35-45 | | |
| **Mean ± SD** | 38.441±3.225 | 39.981±2.324 | 6.437 | 0.758 |
| **After 2 weeks of vitamin D therapy** | | | | |
| **Range** | 35-45 | 35-45 | | |
| **Mean ± SD** | 40.321±3.276 | 40.761±3.135 | 12.328 | 0.431 |

*P. value is significant if < 0.05. P value = comparison between group I and II.

**Table 6. Correlation between serum level of S100-B and both PH and vitamin D in Group I.**

| PH | Serum S100-B | |
|---|---|---|
| Before vitamin D administration | r =- 0.913 | P = 0.001* |
| After 2 weeks of vitamin D administration | r = - 0.858 | P = 0.001* |
| **Serum vitamin D** | **Serum S100-B** | |
| Before vitamin D administration | r = - 0.913 | P = 0.001* |
| After 2 weeks of vitamin D administration | r = - 0.858 | P = 0.001* |

*P value is significant if <0.05.

## 4. DISCUSSION

Neonatal HIE is defined as injury of the immature brain, leading to cell death *via* excite-toxicity, inflammation and oxidative stress. It is the major cause of neonatal mortality and morbidity as a result of permanent neurological disabilities [23]. During HIE, an excessive amount of the excitatory amino acid glutamate is released from presynaptic terminals of nerve cells which is an important neurotransmitter that plays a major role in the development of the central nervous system (CNS) and is likely involved in normal brain functions including cognition, learning, and memory [24].

The aim of this study was to assess the role of vitamin D as an adjuvant therapy for management of 30 neonates with grade II HIE who received single oral daily vitamin D3 of for 2 weeks in addition to daily SC human recombinant erythropoietin for 5 days and magnesium sulphate for 3 days compared with 30 neonates with Sarnat grade II HIE who received daily SC human recombinant erythropoietin for 5 days and magnesium sulphate for 3 days without use of vitamin D.

In the current study, there were no significant differences in serum 25(OH) vitamin D levels between group I and group II before therapy while there were significantly lower serum 25(OH) vitamin D levels in group I and group II compared with controls. This is in agreement with Lowe *et al.* 2017 [25] and Mutl *et al.* 2016 [26] who found significantly lower serum level of vitamin D in the majority of full-term neonates with HIE which may be related to lower circulating anti-inflammatory IL-17.

The decreased level of serum 25(OH) D may be explained by urinary losses of 25(OH) D in HIE infants as HI-induced renal injury is common and involves tubular dysfunction with proteinuria which takes days to resolve. Renal reabsorption of 25(OH) D-bound DBP occurs in proximal tubular cells upon binding to megalin, a transmembrane receptor found on many cell types, including brain capillary endothelial cells, neurons and astrocytes [27]. Ischemia reperfusion down-regulates renal megalin expression [28], which may result in excessive urinary losses of vitamin D [29]. Also HI injury increases the conversion of 25(OH) D to 1, 25(OH) 2D, and both 1, 25(OH) 2D and 25(OH) D may be subjected to increased degradation [30]. Along with increased urinary losses [31], uptake of 25(OH) D into tissues for intracellular production of 1, 25(OH) 2D, may contribute to low 25(OH) D serum concentrations [25].

In this study, S100-B level measured in the first 6 hours after birth before therapy indicated significantly higher serum S100-B levels as a marker of neonatal hypoxia in group I and group II with HIE grade II compared with controls with no significant differences between group I and group II while after therapy, there was significant reduction in S100-B level in group I compared with group II which may indicate the good therapeutic value of vitamin D supplementation in neonates with HIE.

This is in agreement with Chiang *et al* 2015 [32] and Distefano *et al.* 2002 [33] who found significantly higher serum S-100 levels in preterm babies with perinatal asphyxia. Also, Zaigham *et al* 2016 [34] concluded that umbilical cord blood S100-B concentrations are already elevated at birth in asphyxiated newborns developing moderate-severe HIE within a few hours of life, and S100-B concentration is related to the severity of encephalopathy and the risk of developing a permanent sequel. An association between elevated neonatal S100-B levels and development of encephalopathy has been established in several studies with a significant relationship between S100-B levels and severity of HIE and poor neural outcome [8, 35-38].

Increased S100B in HIE may be explained by selective leakage of S100B into the cerebrospinal fluid and then into the blood due to brain injury [39] or by stimulation of secretion of S100B from astrocytes due to metabolic stress [40].

In the present study, early postnatal arterial blood gases show acidosis in group I and II with no significant difference between group I and II in PH, PO2 and PCO2 before therapy but after therapy, there was a significantly higher PH level in group I compared with group II while there were no significant differences between group I and group II regarding PO2 and PCO2. This was in agreement with Chiang *et al.* (2015) [32] and Distefano *et al.* (2002) [33] who found acidosis in newborns with HIE.

There were no significant differences between group I and group II regarding serum IL-17 levels while there were significantly higher serum IL-17 levels in group I and group II compared with controls.

This is in agreement with Verónica *et al.* (2017) [41] who found that the expression and serum levels of the pro-inflammatory cytokines were significantly increased in the children with asphyxia compared with the controls.

Vitamin D deficiency can result in increased Th17 cell activation [42], while vitamin D therapy down-regulates

Th17 activation [43]. In HIE neonates, 25(OH) D levels correlated with circulating Th17 inhibitory cytokines IL-17E and IL-27, but for IL-27, this effect was overridden by hypothermia treatment. Hypothermia up-regulated anti-inflammatory IL-27 production and release from antigen presenting cells in the serum from 36 to72 hours, even while the total circulating leukocyte, neutrophil, lymphocyte, and monocyte counts were decreasing [44].

There were significant negative correlations in group I between serum S100-B and PH before and after 2 weeks of therapy and significant negative correlations between serum level of vitamin D and S100-B before and after 2 weeks therapy.

## CONCLUSION

Vitamin D was found to improve the cases of group I as demonstrated by reduction of the serum level of S100-B after vitamin D therapy.

## RECOMMENDATION

Extensive multicenter studies on a large number of patients with Sarnat grade II HIE with longer duration of follow up to give valid recommendations about the use of vitamin D therapy as an adjuvant therapy in Sarnat grade II HIE.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present study was done after approval from the Ethical Committee of research center in Tanta University, Egypt.

## HUMAN AND ANIMAL RIGHTS

No animals were used in the study. The research was performed in human in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

## CONSENT FOR PUBLICATION

A written consent was taken from parents of studied patients.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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