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Assessment of 25 (OH) Vitamin D in Neonates with Hypoxic Ischemic Encephalopathy

Mohammed Elsayed Hamed [1], Dina Gamal Abd Elhamed [2],
Ansam Mohamed Alshtewi [1*], Nahed Mahmoud Khater [1]
Departments of [1]Pediatrics and [2]Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt
[*]**Corresponding authors:** Ansam Mohamed Alshtewi, **Mobile:** (+20)01095325445, **Email: ansam.alshtewi@gmail.com**

ABSTRACT

Background: Vitamin D is a hormone that affects a wide range of functions within the body. Neonatal hypoxic ischemic encephalopathy (HIE) is a serious disease that may lead to permanent brain injury.

Objective: The present study aimed to study vitamin D status in hypoxic ischemic in encephalopathy.

Patients and methods: A case control study carried out in newborn intensive care unit (NICU) of Zagazig University Children Hospitals. Total number of cases that met the inclusion and exclusion criteria was 49 full term neonates with HIE divided according to Sarnat stages: stage I; 20 full term neonates, stage II; 15 full term neonates and stage III; 14 full term neonates. Cases were compared to 16 healthy controls.

Results: There was a statistical significant increase in Apgar score 1, 5, 10 in control group compared to all cases groups. There were no statistical significant differences between the studied groups in relation to CBC results. There was a statistical significant difference between the different stages of HIE in CRP and pH. There was a statistical significant increase in frequency of hypoxic change in stage III compared to stage II and I. All stages of HIE showed statistical significant increase in frequency of vitamin D deficiency compared to control group. Stage III had statistical significant increase in frequency of vitamin D deficiency compared to stage I and II.

Conclusion: Serum 25(OH) vitamin D insufficiency is present in the majority of term HIE neonates. 25 (OH) vitamin D was significantly deficient in stage III more than stage I and II.

Keywords: CBC, CRP, HIE, Neonates, Serum 25(OH).

INTRODUCTION

Hypoxic ischemic encephalopathy (HIE) is a potentially devastating neonatal brain injury with long-term neurologic effects that affect between 1 and 8/1,000 live birth with the highest rates in developing countries. Hypoxic-ischemic encephalopathy (HIE) is an important cause of acquired neonatal brain injury in term newborn infants and it may lead to neonatal death and long-term disability **(1)**.

Vitamin D is an important neuro-steroid during development and after CNS injury. Deficiency of vitamin D contributes to many diseases that involve systemic or CNS inflammation, and vitamin D deficient adults have worse outcomes after stroke **(2)**. The significance of vitamin D as an immune-modulator and regulator of pro-inflammatory Th17 lymphocytes has been well established in adult stroke. Adult patients have demonstrated an increased proportion of Th17 lymphocytes within 24 hours and one week after stroke **(3)**.

Circulating concentrations of prohormone 25(OH)D are important for the maintenance of CNS concentrations of active 1,25(OH)2 vitamin D (1,25(OH)2D), which is synthesized in many extra-renal cells, including neuronal and glial cells that contain 1-α-hydroxylase. Thus, serum concentrations of 25(OH)D may be crucial for vitamin D's neuroprotective and immune functions after HI injury, in addition to endocrine roles in calcium and phosphorus homeostasis **(4)**.

Most vitamin D studies in neonates have focused on its role in mineral metabolism. Little is known about vitamin D status and immunomodulatory function in neonatal hypoxic-ischemic encephalopathy (HIE) **(5)**. The current study aimed to seek for reducing risk of HIE in neonate**.**

PATIENT AND METHODS

This study was case control study carried out in NICU unit of Zagazig University Children Hospitals. Total number of cases that met the inclusion and exclusion criteria was 49 full term neonates with HIE divided according to Sarnat stages: stage I; 20 full term neonates, stage II; 15 full term neonates and stage III; 14 full term neonates and cases were compared to 16 healthy controls.

Inclusion criteria for studied cases:

Full term neonate's ≥ 37weeks of gestation of any mode of delivery. Both gender who were born with perinatal asphyxia or asphyxia required resuscitation as follow: fetal distress, passage of meconium, metabolic acidosis, and failure to establish spontaneous respiration, depression of Apgar scores, hypoxic ischemic encephalopathy and multiorgan involvement. Neonatal neurologic sequelae (e.g. seizures, hypotonia, coma).

Inclusion criteria for control group: Full term ≥37 weeks of gestation neonate who did not require resuscitation, Apgar score on the first and the fifth minutes of life >8 and no neonatal disease.

Exclusion criteria: Premature infants (gestational age <37 weeks) or post term ≥ 42 weeks. Infants with

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congenital lung pathology or cyanotic congenital heart disease leading to persistent hypoxia, central nervous system malformations, metabolic disorders and maternal anti-epileptic drugs use, maternal drug dependency and maternal analgesics, intracranial hemorrhage, sepsis and genetic disorders and other conditions associated with early neonatal encephalopathy. Neonates with major congenital malformations, chromosomal abnormalities or presence of sepsis.

Hospital protocol was used to manage HIE neonates in NICU. They were given oxygen, intravenous fluids, vitamin K, inotropes (Dopamine and/or Dobutamine each by 1–20 mg/kg/min) and anticonvulsants (Phenobarbitone 20 mg/kg as loading dose, followed by 3–5 mg/kg/d, and phenytoin was also added with same dose in non-responder to phenobarbitone), wherever required.

All cases admitted in newborn intensive care unit (NICU) were subjected to full history, pregnancy details including: blood groups and rhesus incompatibility, history of infertility, maternal medication/drug, trauma / accidents / injury and infection. All studied cases were examined for breathing, circulation and complete physical examination was essential and included: weight, head circumference, sex, temperature on admission, dysmorphic features, meconium staining of skin, organomegaly, head size and shape, fontanelle, bruising or petechiae and presence of seizures.

Measurement of serum 25 (OH) Vitamin D by ELISA:

Serum sample were 1.5 ml of venous blood was withdrawn and divides into 2 vacutainer 0.5 ml in to EDTA vacutainer for complete blood counting and 1ml into plain vacutainer. The blood in plain vacutainer was allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000xg. One tube was used for assay of kidney functions tests, serum electrolytes and serum CRP level and the second table was frozen at -20°C till the assay of serum vitamin D was performed after all samples were collected from all patients and controls .

The definitions of vitamin D deficiency, insufficiency and sufficiency, were as follows: 25 (OH) Vitamin D levels:

1- <10 ng/ml will be defined as 25(OH) Vitamin D deficiency.
2- From 10-30 ng/ml as 25(OH) Vitamin D insufficiency.
3- 30 ng/ml -100 ng/ml as 25(OH) Vitamin D sufficient.
4- >100 ng/ml as intoxication.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Informed consent was obtained from parents or guardians of all participating neonates. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis

All data were analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Qualitative data were expressed as number and percentage and were compared by Chi square test (χ^2) and Fisher exact test as indicated. Quantitative data were expressed as mean ± SD (Standard deviation) and range for parametric and median and interquartile range for non-parametric data. One-way ANOVA, and Kruskal-Wallis test were used. Post Hoc tests: Tukey honestly significant difference test was used as a post hoc test to adjust for multiple comparisons after significant ANOVA test to indicate which significant difference between pairs of groups. All statistical comparisons were two tailed with significance level of P-value ≤ 0.05 indicated significant and p <0.001 indicated highly significant difference.

RESULTS

The present study showed no statistical significance differences between the studied groups in gestational age, birth weight, sex distribution or mode of delivery (Table 1).

Table (1): Birth weight, gestational age, sex, and mode of delivery of the studied groups

Variable		Stage I (n=20)		Stage II (n=15)		Stage III (n=14)		Control (n=16)		P
G. Age (weeks)	Mean ± SD	37.75±0.85		37.53±0.6		37.79±0.89		37.88±0.89		0.70
	Range	37 - 40		37-39		37-40		37-40		
Birth weight (Kg)	Mean ± SD	3.04±0.60		2.72±0.22		3±0.53		2.99±0.23		0.17
Variable		**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**P**
Sex	Female	11	55	6	40	5	35.7	8	50	0.67
	Male	9	45	9	60	9	64.3	8	50	
Mode of delivery	CS	16	80	8	53.3	9	64.3	11	68.8	041
	NVD	4	20	7	46.7	5	35.7	5	31.3	

G: Gestational, SD: Standard deviation

There was a statistical significance increase in Apgar score 1, 5, and 10 in control group compared to all cases groups (Table 2).

Table (2): Apgar score among the studied groups

Variable		Stage I (n=20)	Stage II (n=15)	Stage III (n=14)	Control (n=16)	P
Apgar score 1 min:	Mean ± SD Range Median (IQR)	0.65±0.59 0-2 1 (0-1)	0.33±0.49 0-1 0 (0-1)	8.44±0.51 8-9 8 (8-9)	8.44±0.51 8-9 8 (8-9)	<0.001 **
Within groups:	Versus Stage II Versus Stage III Versus Control	0.11 NS 0.15 NS <0.001**	--- 0.90 NS <0.001**	--- --- <0.001**		
Apgar score 5 min:	Mean ± SD Range Median (IQR)	1.55±0.69 0-3 2 (1-2)	1.33±0.62 1-3 1(1-2)	1.43±0.65 1-3 1(1-2)	9±0 9 9(9-9)	<0.001 **
Within groups:	Versus Stage II Versus Stage III Versus Control	0.21 NS 0.45 NS <0.001**	--- 0.63 NS <0.001**	--- --- <0.001 **		
Apgar score 10 min:	Mean ± SD Range Median (IQR)	2.8±0.77 1-4 3 (2-3)	2.2±1.08 1-4 2 (1-3)	2.29±0.83 1-4 2 (2-3)	9.81±0.4 9-10 10 (10-10)	<0.001 **
Within groups:	Versus Stage II Versus Stage III Versus Control	0.05 NS 0.06 NS <0.001**	--- 0.61 NS <0.001**	--- --- <0.001**		

SD: Standard deviation, IQR: Interquartile range, **: Highly Significant

There were no statistical significant differences between the studied groups in relation to CBC results (Table 3).

Table (3): CBC findings of the studied groups

Variable		Stage I (n=20)	Stage II (n=15)	Stage III (n=14)	Control (n=16)	P
Hb (gm/dl)	Mean ± SD	15.84±2.86	16.42±4.42	16.31±3.68	16.3±1.69	0.95
WBCs ($x10^3/mm^3$)	Mean ± SD	11.83±2.46	15.53±3.95	13.24±2.94	12.74±3.35	0.42
Platelets ($x10^3/mm^3$)	Mean ± SD	207.55±6.51	189.78±9.33	230.29±9.81	238.69±5.6	0.10
MCV	Mean ± SD	93.56±2.28	97.44±8.26	91.61±13.81	97.64±3.08	0.60
MCH	Mean ± SD	35.17±2.65	33.85±3.2	34.06±1.82	33.98±1.43	0.33

SD: Standard deviation

There was a statistical significant difference between the different stages of HIE in CRP and pH. Comparing each two groups, it was founded that stage III was statistically higher than stage I and II also pH was statistically lower in stage III compared to stage II. No difference was found between different stages of HIE in PCO_2, PO_2 or HCO_3 (Table 4).

Table (4): CRP and ABG among neonate with HIE

Variable		Stage I (n=20)	Stage II (n=15)	Stage III (n=14)	P
CRP (mg/dl)	Mean ± SD	12.71±2.96	5.91±1.19	29.98±4.59	<0.001**
pH	Mean ± SD	7.11±0.23	7.22±0.09	6.96±0.45	0.06
PCO_2	Mean ± SD	43.85±5.33	44.21±8.86	45.06±3.25	0.96
PO_2	Mean ± SD	68.21±6.60	66.33±2.52	68.24±9.38	0.65
HCO_3	Mean ± SD	14.33±2.36	14.16±3.83	12.64±3.39	0.44

SD: Standard deviation, *: Significant **: Highly Significant. P1: Stage I versus II P2: Stage I versus III P3: Stage II versus III.

There was a statistical significant increase in frequency of hypoxic change in stage III compared to stage II and I. There was a statistical significant increase in frequency of death among stage III compare to Stage I and II (Table 5).

Table (5): Radiological findings and fate among neonate with HIE

Variable		Stage I (n=20)		Stage II (n=15)		Stage III (n=14)		P
		No	%	No	%	No	%	
CT or MRI	Normal (n=52)	18	90	11	73.3	7	50	**0.03***
	Hypoxic change (n=13)	2	10	4	26.7	7	50	
Fate	Survived (n=51)	19	95	11	73.3	5	35.7	**<0.001**
	Dead (n=14)	1	5	4	26.7	9	64.3	**

*: Significant, ** Highly significant

All stages of HIE showed statistical significant increase in frequency of vitamin D deficiency compared to control group. Stage III had statistical significant increase in frequency of vitamin D deficiency compared to stage I and II (Figure 1, 2).

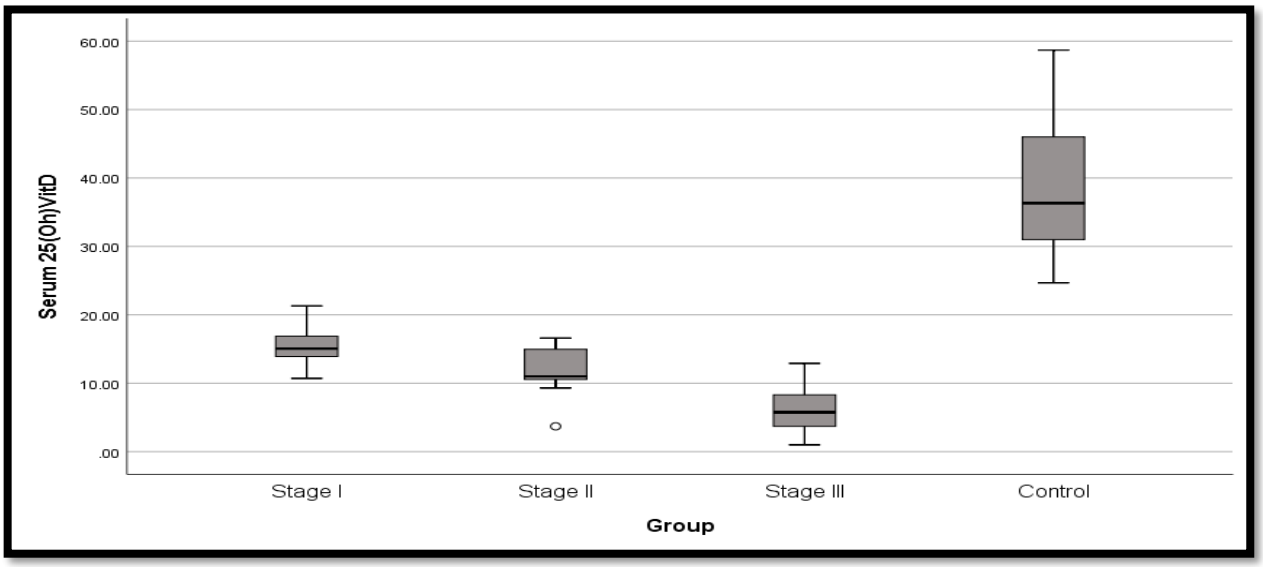

Figure (1): Serum 25(OH) vitamin D level among the neonates with HIE groups

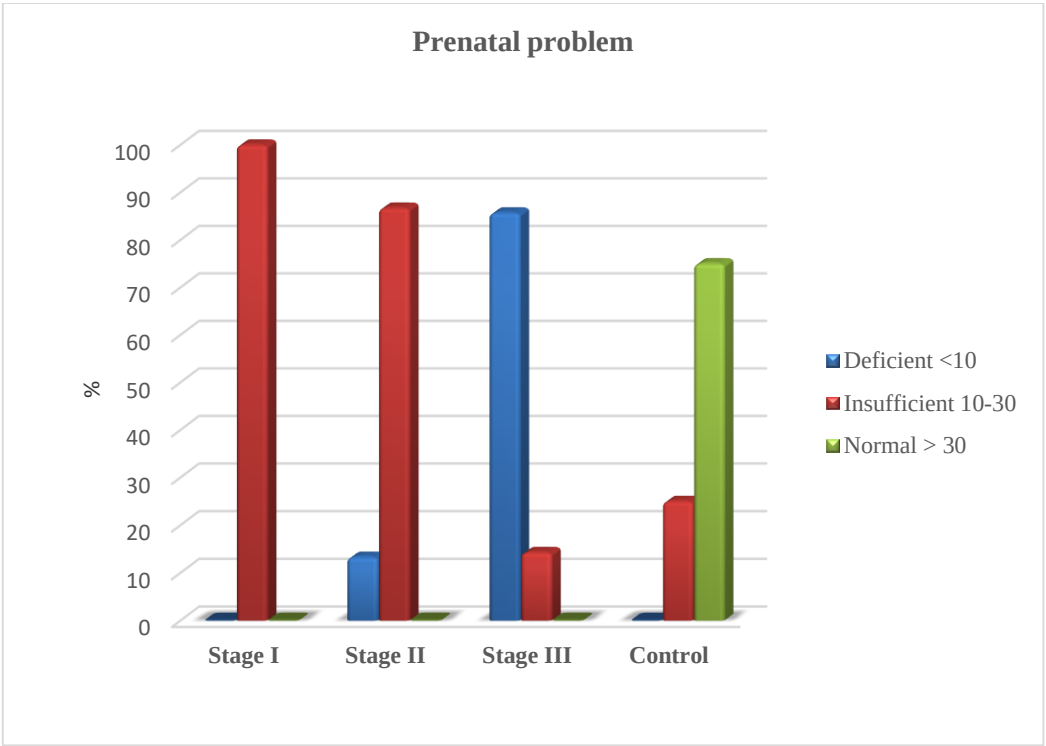

Figure (2): Frequency of Serum 25(OH) vitamin D deficiency among the studied groups

DISCUSSION

The significance of vitamin D as an immunomodulator and regulator of pro-inflammatory Th17 lymphocytes has been well established in adult stroke. Adult patients have demonstrated an increased proportion of Th17 lymphocytes within 24 hours and one week after stroke. These findings may be pertinent to neonatal HI, as naïve T cells develop into Th17 cells more readily in infants than adults, and may contribute to neonatal inflammatory response to HI injury. Vitamin D has been shown to reduce pro-inflammatory Th17 differentiation and proliferation, and IL-17 cytokine production, while promoting anti-inflammatory IL-10 and T regulatory cells [(6)].

However, vitamin D degradation is increased in neuroinflammation, which may limit its effect as a Th17 immunomodulator after HI. In addition, vitamin D deficiency (<20 ng/ml) and insufficiency (<30 ng/ml) is widespread in human neonates. In the only other report on vitamin D status in neonatal HIE, **Mutlu *et al.*** demonstrated lower serum 25(OH) vitamin D (25(OH)D) concentrations in 31 cooled HIE infants in Turkey compared with healthy term control infants. In this study all HIE infants had serum 25(OH)D < 20 ng/ml on day of life 1, and 30% infants had persistently low serum 25(OH)D on day 5 [(7)].

Circulating concentrations of prohormone 25(OH)D are important for the maintenance of CNS concentrations of active 1,25(OH)2 vitamin D (1,25(OH)2D), which is synthesized in many extra-renal cells, including neuronal and glial cells that contain 1-α-hydroxylase. Thus, serum concentrations of 25(OH)D may be crucial for vitamin D's neuroprotective and immune functions after HI injury, in addition to endocrine roles in calcium and phosphorus homeostasis [(8)].

The main aim of this study was to reduce the risk of HIE in neonate. This study was case control study carried out in NICU of Zagazig University Children Hospitals. Total number of cases that met the inclusion criteria was 49 full term neonates with HIE divided according to Sarnat stages: stage I; 20 full term neonates, stage II; 15 full term neonates and stage III; 14 full term neonates. Cases were compared to 16 healthy controls.

The main results of this study were as follows:

There were no statistical significance differences between the studied groups in sex distribution. Our results were in agreement with study of **Mutlu *et al.*** [(7)] as they reported that there were no statistical significant differences between the studied groups in sex distribution. Hypoxic ischemic encephalopathy (HIE) is a potentially devastating neonatal brain injury with long-term neurologic effects that affects between 1 and 8/1,000 live births with the highest rates in developing countries [(9)].

The present study showed that there were no statistical significant differences between the studied groups in gestational age, birth weight and mode of delivery but there was a statistical significant lower Apgar score at 1, 5, 10 minute in cases compared to control groups. Our results were in agreement with study of **Saleh *et al.*** [(1)] as they revealed that there is significant difference between case group and control group regarding Apgar score at 1.5 minute as all case group patients had Apgar score less than 7 as suggested in the inclusion criteria. On the other hand, control group had Apgar score more than 8 at 1.5 minutes which is also in agreement with study of **Calvert and Zhang** [(10)].

There were no statistical significant differences between the studied groups in CBC, kidney function tests (KFTs) or electrolyte level. There was a statistical significant difference between the studied cases groups in CRP and pH. Comparing each two groups, it was found that CRP in stage III was statistically higher than stage I and II and pH was statistically less in stage III compared to stage II. No difference was found between cases groups in PCO_2, PO_2 or HCO_3. Our results are in agreement with study of **Mutlu *et al.*** [(7)] as they reported that in the HIE group, blood gases and pH levels were statistically significantly lower compared with the control group. CRP levels were significantly high in the HIE group. The inflammation has an important role in the pathogenesis of neonatal brain damage. The level of CRP increases when there is an inflammation throughout the body. CRP levels were significantly high in the HIE group. A positive correlation between HIE stages and CRP levels suggest that there was an inflammation in the infants with HIE. Active form of vitamin D has anti-inflammatory and immunomodulatory effects and vitamin D is important for the production of cathelicidin a natural antimicrobial peptide produced within macrophages. In our cases there was no significant correlation because there was no infection.

In our study there was significant difference between studied groups in relation to radiological findings (CT or MRI); in stage III (50%) were higher than that of stage II (26.7%) and I (10%). There was a statistical significance increase in frequency of death among stage III compared to stage I and II. In agreement with our results, the study of **Saleh *et al.*** [(1)] revealed that there was significant difference between case group and control group regarding CT finding. On other studies there were 76% of their studied cases had a positive finding in CT ranging in severity from brain edema in 20% of cases to hypoxic changes as hypodensity in CT findings in 56.7% of them, which is in agreement with others [(11, 12)].

Our results agreed with **Lowe *et al.*** [(5)] who revealed that 25(OH) vitamin D insufficiency was present in the majority of their HIE infants, and half of these infants had decreasing 25(OH)D over the initial 72h of treatment, in spite of administration of 417 ng/h ergocalciferol in total parentral nutrition (TPN), which

did not appreciably increase serum 25(OH)D concentrations.

Also, **Zhang *et al.* [13]** stated that vitamin D is a well-known regulator of inflammatory T cells, and inhibits Th17 cell activation. Another anti-inflammatory cytokine that may oppose Th17-induced inflammation is IL-27 were also correlated with 25(OH)D levels in normothermic HIE infants, providing additional evidence that vitamin D insufficient infants may have limited ability to mitigate post-HI Th17 inflammation.

Serum 25(OH)D also serves as the main source for local CNS production of 1,25(OH)2D. Serum 25(OH) vitamin D concentrations are known to decrease after stroke in adults. Although serum 25(OH)D has a two-week half-life under physiologic conditions, inflammation contributes to accelerated depletion of serum 25(OH)D, which in turn limits 1,25(OH)2D production by leukocytes and neural cells. The net effect of increased vitamin D metabolism in inflamed CNS tissue is to reduce the circulating substrate, 25(OH)D, while circulating 1,25(OH)2D may initially be more stably maintained [14].

CONCLUSION

Serum 25(OH) vitamin D insufficiency is present in the majority of term HIE neonates, 25 (OH) vitamin D was significantly deficient in stage III more than stage I and II. More studies are needed on mothers to evaluate vitamin D and compare it with neonatal vitamin D.

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