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EFFECTS OF HYPOXIA-ISCHEMIA ON 25 (OH) VITAMIN D STATUS IN NEONATES WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY

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ABSTRACT

Background: 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**.** Little is known about vitamin D status, immuno-modulatory function, or effects of hypothermia on vitamin D binding protein (DBP) in neonatal hypoxic-ischemic encephalopathy (HIE). The current study was aimed to reduce risk of HIE in neonate**.**

Subjects and methods: A 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 termneonates and stage III; 14 full term neonates, was compared to16 healthy controls. All cases admitted in NICU were subjected to full history, pregnancy details. Measurement of serum 25 (OH) Vitamin D was performed using ELISA.

Results: There was a statistical significant increase in frequency of hypoxic change in studied cases compared to control group. there was a statistical significant increase in frequency of hypoxic change in stage III compared to stage II &I. finally there was a statistical significant increase in frequency of death among Stage III compare to Stage I & II. All stages of HIE showed statistical significant increase in frequency of Vit D deficiency comparing to control group stage III had statistical significant increase in frequency of Vit D deficiency comparing to stage I & II.

Conclusion: Serum 25(OH) vitamin D insufficiency is present in the majority of term HIE neonates, 25 (OH) Vit D was significantly deficient in stage III more than stage I & II. There was a statistical significant deficiency Vit D level among dead cases compared to survived cases.

Keywords: Serum 25(OH); HIE; Apgar score; Vit D deficiency

INTRODUCTION

Hypoxia-ischemia initially causes energy failure and loss of mitochondrial function. This is accompanied by membrane depolarization, brain edema, an increase of neurotransmitter release and inhibition of uptake, and an increase of intracellular calcium that sets off additional pathologic cascades **(1)**.

Hypoxic ischemic encephalopathy is the hypoxic ischemic brain injury as a result of perinatal asphyxia, Hypoxic ischemic encephalopathy (HIE) is the most important consequence of perinatal asphyxia and it is one of the serious neurologic problems of the perinatal period. Also, HIE is the single most important perinatal cause of neurological morbidity in both full –term and premature infant **(2)**.

Vitamin D has neuroprotective and immunomodulatory properties, and deficiency is associated with worse stroke outcomes. Little is known about effects of hypoxia–ischemia or hypothermia treatment on vitamin D status in neonates with hypoxic–ischemic encephalopathy (HIE). We hypothesized vitamin D metabolism would be dysregulated in neonatal HIE altering specific cytokines involved in Th17 activation, which might be mitigated by hypothermia **(3)**.

However, vitamin D degradation is increased in neuro-inflammation, which may limit its effect as a Th17 immuno-modulator 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 neonates **(4).**

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

Subjects and methods:

This study was case control study carried out in NICU unit of Zagazig University Children Hospitals. The study was approved by the ethics committees of the university. Informed consent was obtained from parents or guardians of all participating neonates

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, was 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 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, multiorgan involvement and 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 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 and also neonates with major congenital malformations, chromosomal abnormalities or presence of sepsis.

Clinical Assessment:

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 1–20mg/kg/min) and anti-convulsants (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 NICU were subjected to full history, pregnancy details including: blood groups and rhesus incompatibility, history of infertility, maternal medication and infection. All studied cases were examined for breathing, circulation and complete physical examination is essential and include: 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.

Investigations:

Laboratory investigations including complete blood picture, blood gases, pH determination with calculation of base deficit

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 0.5ml in to EDTA vacutainer for complete blood counting and 1ml into plain vacutainer The blood in plain vacatianer was allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000xg. The kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principal of competitive binding. The absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional to the amount of 25-OH Vitamin D in the sample, the assay measures both the 25-OH Vitamin D2 and D3. The total assay procedure run time is 75 minutes.The definitions of of Vitamin D deficiency, insufficiency and sufficiency, were as follows: 25 (OH) Vitamin D levels: 10 ng/ml will be defined as 25(OH) Vitamin D deficiency, From 10-30 ng/ml as 25(OH) Vitamin D insufficiency, 30 ng/ml -100ng/ml as 25(OH) Vitamin D suffient and 100ng/ml as intoxication.

Statistical analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 27.0. Qualitative data were represented as frequencies and relative percentages. Chi square test was used to calculate difference between qualitative variables. All statistical comparisons were two tailed with significance Level of P-value ≤

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0.05 indicates significant, p <0.001 indicates highly significant difference while, P> 0.05 indicates non-significant difference.

RESULTS

The present study showed no statistical significance differences between the studied groups in sex distribution **(Figure 1)**. There was no statistical significance differences in Apgar score at (T10) in control group compare to all cases groups **(Figure 2)**. There was a statistical significant increase in frequency of hypoxic change in studied cases compared to control group. there was a statistical significant increase in frequency of hypoxic change in stage III compared to stage II &I. finally there was a statistical significant increase in frequency of death among Stage III compare to Stage I & II **(Table 1)**. All stages of HIE showed statistical significant increase in frequency of Vit D deficiency comparing to control group stage III had statistical significant increase in frequency of Vit D deficiency comparing to stage I & II**(Table 2)**. There was a statistical significance decrease in level of 25 (OH) Vitamin D among dead cases compared to survived cases **(Figure 3)**.

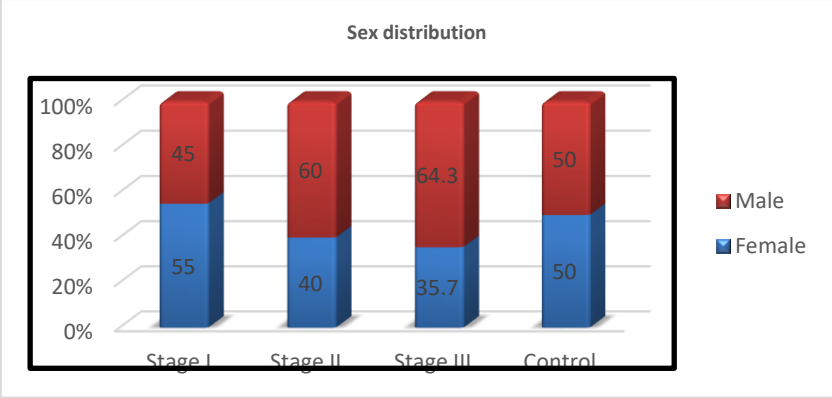

Figure (1): Sex distribution of the neonatal with HIE groups

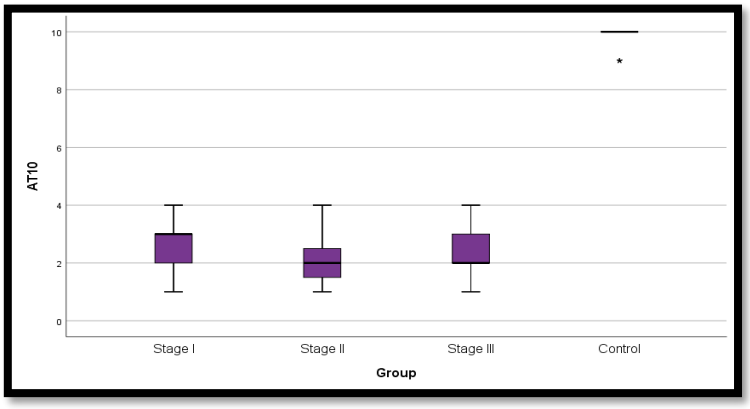

Figure (2): Apgar score at (T10) among the neonatal with HIE groups.

Table (1): Radiological findings and fate among the cases groups:

Variable		Stage I (*n*=20)		Stage II (*n*=15)		Stage III (n=14)		χ^2	P
		No	%	No	%	No	%		
CT or MRI:	*Normal*	17	85	10	66.7	12	85.7	2.23	0.33
	Hypoxic change	3	15	5	33.3	2	14.3		NS
Fate:	*Survived*	19	95	11	73.3	5	35.7	**14.22**	**0.001**
	Dead	1	5	4	26.7	9	64.3		*

χ^2: Chai square test. NS: Non significant (P>0.05) ***: Significant (P<0.05)**

Table (2): Serum level of 25 (OH) Vitamin D among the neonatal with HIE groups:

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Variable		Stage I (*n*=20)		Stage II (*n*=15)		Stage III (n=14)		Control (n=16)		KW	P
25 (OH):	*Mean ± SD* *Range* *Median(IQR*	15.28±2.53 10.7-21.3 15.05(13.9-17)		12.11±3.38 3.7-16.6 11(10.5-15)		6.02±3.38 1-12.9 5.75(3.46-8.58)		38.16±10.13 24.68-58.69 36.33(30.5-47)		52.34	<0.001 **
Within groups:	*Versus Stage II* *Versus Stage III* *Versus Control*	**0.006*** **<0.001**** **<0.001****		--- **<0.001**** **<0.001****		-- --- **<0.001****					
Variable		**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**χ^2**	**P**
25(OH):	Deficient <10 Insufficient 10-30 Normal > 30	0 20 0	0 100 0	2 13 0	13.3 86.7 0	12 2 0	85.7 14.3 0	0 4 12	0 25 75	**87**	**<0.001 ****
Within groups:	*Versus Stage II* *Versus Stage III* *Versus Control*	0.09 NS **<0.001**** **<0.001****		--- **<0.001**** **<0.001****		--- --- **<0.001****					

SD: Stander deviation, IQR: interquartile range KW: Kruskal Wallis test χ^2: Chai square test NS: Non significant (P>0.05) *: Significant (P<0.05) **: Highly Significant (P<0.001).

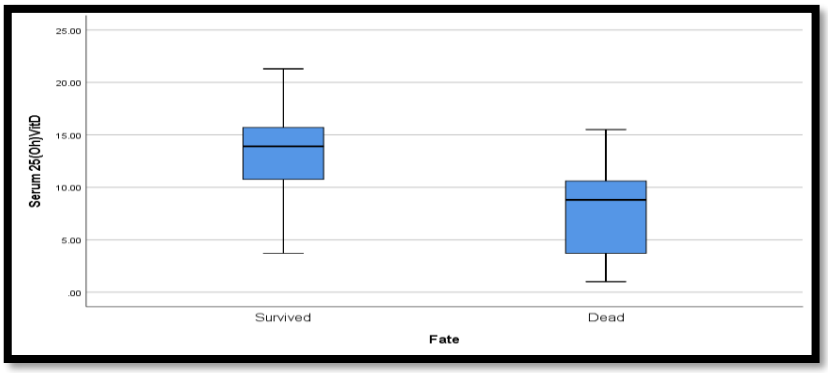

Figure (3): Relation between Serum 25(OH) and fate among the cases groups.

DISCUSSION

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. Energy failure, membrane depolarization, edema, an increase of neurotransmitter release, the inhibition of neurotransmitter uptake, an increase of intracellular Ca2+, the production of oxygen-free radicals (OFR), lipid peroxidation, and a decrease of blood flow all contribute to the progression of the brain damage that subsequently follows a hypoxic–ischemic insult **(5).**

Vitamin D is an important neurosteroid during development and after CNS injury. Deficiency of vitamin D contributes to many diseases that involve systemic or CNS inflammation, and vitamin D deficient in adults have worse outcomes after stroke. Most vitamin D studies in neonates have focused on its role in mineral metabolism. Little is known about vitamin D status, immunomodulatory function in neonatal hypoxic-ischemic encephalopathy (HIE) **(6)**.

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 **(6)**.

In our study was incidence of cesarean section in the study group stage I (80%), stage II (53.3%), stage III (64.3%) and control group (68.8%).However, in **Mutlu et al., (7)** study showed the incidence of cesarean section was high in both the control (42%) and study groups (57%). Primary cesarean section in the study group was 54% (n, 7) and six of these mother VD levels were between 5 and 15 ng/mL and the other were 55 ng/mL. In the control group, primary cesarean section was 35% (n= 6) and two of them VD levels were between 15 and 20 ng/mL and the others were between 5 and 15 ng/mL.

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Perinatal asphyxia and secondary hypoxic-ischemic encephalopathy (HIE) are an important cause of acquired neonatal brain injury in term newborn infants and it may lead to neonatal death and long-term disability. Energy failure, membrane depolarization, edema, an increase of neurotransmitter release, the inhibition of neurotransmitter uptake, an increase of intracellular Ca2+, the production of oxygen-free radicals (OFR), lipid peroxidation, and a decrease of blood flow all contribute to the progression of the brain damage that subsequently follows a hypoxic–ischemic insult. Human body contains various antioxidant defense mechanisms to prevent the damage from Oxygen free radical with intrinsic (e.g., superoxide dismutase (SOD), catalase, glutathione peroxidase (GP), glutathione) and extrinsic (e.g., vitamin E and vitamin C) antioxidants (**8).**

Neuroimaging using magnetic resonance imaging, conventional, diffusion, and spectroscopy from 24 h to 96 h of life gives a useful guide regarding the potential timing of a cerebral insult especially diffusion abnormalities. In Neonatal encephalopathy (NE) associated with an acute event (HIE), the typical pattern of injury on MRI showed involvement of basal ganglia, thalami with associated posterior limb of the internal capsule and in severe cases brain stem involvement (**10).**

Vitamin D a key nutrient for children's well-being and growth, is essential for bone health and may contribute to other health benefits. Vitamin D deficiency is a worldwide problem that has been associated with wide spectrum of diseases, ranging from neurological disorders to chronic inflammatory conditions **(11).**

In the present study we found that stage III had statistical significant increase frequency in Vit D deficiency comparing to stage I & stage II as there is (85.7%) had deficient level Vit D and (14.3%) had insufficient level of Vit D, stage II is (13.3%) had deficient level of Vit D, (86.7%) had insufficient level of Vit D and stage I had (100%) insufficient level of Vit D.

Our results were in line with study of **Saleh et al., (11)** reported that hypoxic ischemic encephalopathy had a significant lower vitamin D than controls as there is 50% had a deficient level of vitamin D and 23.3% had insufficient level of vitamin D, and 26.7% had a normal level of vitamin D in comparison with control group which had normal level in 90% and only 10% had insufficient level which is in agreement with **Kurinczuk et al., (12) and Mughal, (13).**

The current study showed that there was no statistical significant correlation between serum 25 (OH) vit D and any of the studied parameters (sex, radiological findings) among the studied cases groups. There was a statistical significance decrease in Vit D level among dead cases compared to survived cases.

Our results agree with study of **Saleh et al., (11)** as they reported that there was no significant correlation between neonatal serum levels of vitamin D and gender, mode of delivery, consanguinity or residence in both cases and control groups (**14).**

According to **Mutlu et al., (7) revealed** the correlation analysis between HIE stages and Vit D levels measured on day 1 and day 5 did not demonstrate a significant correlation.

CONCLUSION

Serum 25(OH) vitamin D insufficiency is present in the majority of term HIE neonates, 25 (OH) Vit D was significantly deficient in stage III more than stage I & II. There was a statistical significant deficiency Vit D level among dead cases compared to survived cases.

No Conflict of interest.