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Vitamin D and Hyperbilirubinaemia in Neonates

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Abstract ***Objectives:*** Low antioxidant system may contribute to the severity of neonatal hyperbilirubinaemia. This study was performed with the aim of establishing whether there is a relationship between serum vitamin D level and the hyperbilirubinaemia in full-term neonates. ***Material and methods:*** This prospective study was performed by comparing serum vitamin D levels in newborns with a pathological level of hyperbilirubinaemia and healthy newborns with a physiological level of hyperbilirubinaemia or without jaundice. Ethical committee approval was obtained and written informed consent forms were received from babies' families. ***Results:*** A statistically significant difference was found in the serum 25-hydroxy vitamin D levels between newborns with hyperbilirubinaemia and control group (p=0.01). A significant negative correlation was noted between serum vitamin D and serum parathyroid hormone levels among the neonates recruited (r:-0.3, p:0.03). ***Conclusions:*** Our results suggest that low level of serum vitamin D may associate with hyperbilirubinaemia in full-term neonates.

Key words Hyperbilirubinaemia; Neonate; Vitamin D

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Introduction

Physiological jaundice is frequently encountered in the newborn period. While the causes such as blood group incompatibility, sequestration or polycythaemia can be readily determined but majority have no definite causes identified. Research in recent years identified vitamin D receptors in cells derived from different tissues such as liver, pancreas, brain and prostate. They are also found on surface of immune cells including lymphocytes and macrophages. The potential extra-osseous effects have also been reported.[1-5] On the other hand, vitamin D synthesis begins with the effect of solar rays on skin tissue, and active vitamin D synthesis takes place with 25-hydroxylation in the liver first and finally 1-hydroxylation in the kidney.[4,5] In addition, while liver tissue is involved in vitamin D synthesis, it also plays an important role in the conversion of indirect bilirubin into direct bilirubin. There has been no study investigating the role of vitamin D in hyperbilirubinaemia. Our aim in this study was to

investigate whether there is an association between indirect hyperbilirubinaemia and serum vitamin D levels in newborns with jaundice at a level necessitating phototherapy.

Material and Methods

This prospective study was performed with the aim of determining whether there is an association between serum indirect bilirubin and serum vitamin D levels in newborns with jaundice at a level necessitating phototherapy between December 2010 and March 2011. Ethical committee approval was obtained and written informed consent forms were received from babies' families.

Newborns with postnatal ages of 3-10 days and gestational ages of 37-40 weeks, with a bilirubin level above the pre-set threshold for phototherapy as recommended by the American Academy of Pediatrics.[6] There should be no pathology that accounts for hyperbilirubinaemia such as isoimmunisation, infection, red cells sequestration or polycythaemia. These groups of neonates were enrolled in the study (Group 1). Normal healthy newborns with similar demographic characteristics but no jaundice or just with physiologic jaundice were enrolled in the control group (Group 2). These two groups were recruited simultaneously in the study period. All the newborns included in the study were evaluated in terms of their birth weight, gestational and postnatal age, and body weight on enrolment in the study. Type of delivery, sex and form of feeding were also captured. In addition, the mothers of all the newborns were asked about their age, type of clothing worn, geographical region they lived in, vitamin D use during pregnancy and any disease or drug use that might affect vitamin D levels. Babies whose mothers had a history of chronic liver disease, kidney disease or on regular anti-epileptic drug were excluded.

Haemogram, peripheral smear, reticulocyte count, blood group, direct Coombs test, bilirubin, free thyroxin (FT4), thyrotropin-stimulating hormone (TSH), calcium (Ca), phosphate (P), alkaline phosphatase (ALP), magnesium (Mg), parathyroid hormone (PTH) and 25-hydroxy vitamin D (25-OH vitamin D) levels were investigated in all newborns enrolled, together with mothers' blood groups and Ca, P, ALP, Mg, PTH and 25-OH vitamin D levels.

Subjects with a serum 25-OH vitamin D level greater than 20 ng/mL were regarded as normal, those between 15 and 20 ng/mL as having vitamin D insufficiency, those with values <15 ng/mL as having vitamin D deficiency and those with <5 ng/mL as having severe vitamin D deficiency.[5,7]

Vitamin D and PTH levels were assessed using electrochemiluminescence immunoassay (ECLIA) on a Modular Analystics E170 device and a Roche Elesysve Cobase Roche (Switzerland-2011) kit for immunological testing. Ca, P, Mg and ALP levels were measured using the spectrophotometric technique on a COBAS-C 6000 series auto-analyser device with Cobas (Switzerland-2010) commercial kits.

Statistical Analysis

SPSS 17 was used for statistical analysis. Variables determined by measurement are expressed as mean± standard deviation (min-max), and those determined arithmetically as percentages. Student's t-test was used to compare variables determined by measurement between the two groups, and the chi square or Fisher's exact tests to compare those determined arithmetically. Pearson correlation test was used for correlation. Significance was set at $p<0.05$.

Results

This study recruited 51 newborns; including a 30-member hyperbilirubinaemia group (Group 1) and 21-member control group (Group 2). 1:1 case control study was planned but some families at control arm declined to participate during the study period, therefore number of Group 2 was lower than Group 1. The socio-demographic characteristics of the cases included are shown in Table 1. No statistically significant difference was found between the groups in terms of mean gestational ages (38.2±1.3 and 38.4±0.7 weeks, respectively), birth weight (2989±375 and 3256±507 g), postnatal age (5.7±2.0 and 6.7±2.3 days), body weight at time of study (2987±389 and 3243±506), type of delivery or gender ($p>0.05$) (Table 1). All of these newborns were breastfed. No statistical difference was noted between the two groups in terms of mothers' way of dressing or vitamin D intake ($p>0.05$) (Table 1). All of the newborns (both groups) were wrapped up and nursed by their mothers.

Laboratory parameters between the hyperbilirubinaemia and control groups are shown in Table 2. Bilirubin levels were significantly higher in Group 1 compared to the controls ($p=0.001$). While no statistically significant difference was observed between the groups in terms of serum Ca, P, Mg, ALP levels and white blood cell (WBC) count ($p>0.05$), a significant difference was found in serum 25-OH vitamin D levels ($p=0.01$) (Table 2). A statistically

significant negative correlation was identified between serum vitamin D and PTH levels in the study group (r:-0.3, p:0.03). The serum 25-OH vitamin D deficiency level in the babies in Group I was as high as 86%, with 7% reaching insufficiency level. The vitamin D deficiency level in Group I was statistically significantly more severe than that in Group 2 (p:0.03). No difference was determined between the groups in terms of vitamin D insufficiency ($p<0.05$).

Laboratory parameters on mothers' status between Group 1 and Group 2 are shown in Table 3. There were no statistically significant differences for Ca, P, Mg, ALP, PTH and 25-OH vitamin D levels between the mothers of the hyperbilirubinaemia and control group ($p>0.05$) (Table 3).

Discussion

The 25-hydroxylation stage, one of the important phases of vitamin D synthesis, takes place in the liver, as does bilirubin conjugation. Bilirubin is the final product of heme catabolism. The enzymes heme oxygenase and biliverdin reductase play a role in the conversion of heme into bilirubin. The bilirubin that forms is made soluble in water by being conjugated with the catalyser effect of the enzyme uridine diphosphoglucuronosil transferase present in the endoplasmic reticulum of the hepatocytes. The conjugated bilirubin is released into the bile canaliculi with active transport.[8] At the same time, the bilirubin has an antioxidant property.[8-10]

Vitamin D synthesis largely begins with the direct effect of solar rays on 7 dihydrocholecalciferol in the skin tissue. The cholecalciferol first synthesised in the skin thus turns into 25-OH vitamin D with 25-hydroxylase enzyme in the liver tissue and finally turns into active vitamin D exhibiting its effect in the target organ through the enzyme 1 alpha hydroxylase in the kidney.[11] Although bilirubin and vitamin D metabolisms are 2 distinct pathways and are very different, at least one part of the synthesis takes place in a common organ, the liver. Therefore, the metabolism or synthesis of one may affect the other.

In vitamin D insufficiency or deficiency, serum Ca levels may be low or normal, phosphate levels normal or low, ALP normal or high and PTH level normal or high.[5,7] There is a statistically significant negative correlation between

Table 1 Demographic characteristics of the cases [mean±SD (min-max)]

	Study group (n=30)	Control group (n=21)	P
Mother age	26.9±5.4 (19-39)	26.3±5.5 (19-36)	>0.05
Gestational age	38.2±1.3 (37-40)	38.4±0.7 (37-40)	>0.05
Delivey type			
SVD [n (%)]	17 (57)	16 (76)	>0.05
C/S [n (%)]	13 (43)	5 (24)	>0.05
Male/Female (n)	19/11	12/9	>0.05
Birth weight (gr)	2989±375 (2550-4250)	3256±507 (2150-4300)	>0.05
Postnatal weight (gr)	2987±389 (2420-4000)	3243±506 (2220-4200)	>0.05
Postnatal age (d)	5.7±2.0 (3-9)	6.7±2.3 (3-10)	>0.05
Breast feeding [n (%)]	30 (100)	21 (100)	>0.05
Mothers' dressing type			
Group 1 [n (%)]*	18 (60)	12 (57)	>0.05
Group 2 [n (%)]**	10 (33)	8 (38)	>0.05
Group 3 [n (%)]***	2 (7)	1 (5)	>0.05
Mothers' vitamin D use			
Regularly [n (%)]	2 (7)	3 (14)	>0.05
Irregularly [n (%)]	16 (53)	10 (48)	>0.05
None [n (%)]	12 (40)	8 (38)	>0.05

SVD: Spontaneous vaginal delivery; C/S: Cesarean section

*Group1: Dressed in traditional Islamic style, covering the whole body including hands and face

**Group 2: Traditional clothing with the skin of the hands and face uncovered

***Group 3: Dressed in a style which exposed the usual areas of the skin to sunlight

Table 2 Laboratory parameters measured in the study and control groups [mean±SD (min-max)]

	Study group (n=30)	Control group (n=21)	P
Bilirubin (mg/dL)	19.7±2.3 (15.2-24.9)	6.1±4.3 (0.5-12.0)	0.001
WBC	12.3±2.6 (10.8-16.7)	12.8±3.1 (9.8-17.9)	>0.05
Ca (mg/dL)	9.4±0.8 (8.0-10.8)	9.8±0.7 (8.0-10.7)	>0.05
P (mg/dL)	5.2±1.1 (2.8-8.7)	5.6±1.3 (3.1-7.7)	>0.05
ALP (U/L)	542±182 (158-873)	452±159 (283-697)	>0.05
Mg (mg/dL)	1.9±0.2 (1.7-2.2)	1.9±0.2 (1.6-2.3)	>0.05
PTH (pg/mL)	51.4±26.2 (22-129)	38.7±18.3 (9-95)	>0.05
25-OH vit D (ng/mL)	10.7±4.9 (5.1-27.4)	15.7±4.9 (5-23)	0.01
25-OH vit D			
<5 ng/mL [n (%)]	–	–	
5-14.9 ng/mL [n (%)]	26 (86)	9 (43)	0.03*
15-20 ng/mL [n (%)]	2 (7)	7 (33)	0.60**
>20 ng/mL [n (%)]	2 (7)	5 (24)	

WBC: white blood cell; Ca: calcium; P: phosphate; ALP: alkaline phosphatase; Mg: magnesium; PTH: parathyroid hormone; 25-OH vit D: 25-hydroxy vitamin D

*5-14.9 and >20; **15-20 and >20

Table 3 Laboratory parameters of the study and control group mothers' [mean±SD (min-max)]

	Study group (n=30)	Control group (n=21)	P
Ca (mg/dL)	9.6±0.8 (8.2-10.9)	9.4±1.0 (8.1-10.8)	>0.05
P (mg/dL)	4.7±1.3 (2.8-8.6)	5.2±1.0 (2.4-7.2)	>0.05
ALP (U/L)	344±178 (156-762)	292±140 (89-613)	>0.05
Mg (mg/dL)	1.8±0.3 (1.6-2.1)	1.8±0.2 (1.7-2.3)	>0.05
PTH (pg/mL)	44.5±36.1 (15-190)	38.9±17.6 (13-78)	>0.05
25-OH vit D (ng/mL)	17.2±9.1 (3-46)	17.2±7.3 (7-31)	>0.05
25-OH vit D			
< 5 ng/mL [n (%)]	–	–	
5-14.9 ng/mL [n (%)]	15 (50)	9 (43)	>0.05
15-20 ng/mL [n (%)]	8 (27)	7 (33)	>0.05
>20 ng/mL [n (%)]	7 (23)	5 (24)	>0.05

Ca: calcium; P: phosphate; ALP: alkaline phosphatase; Mg: magnesium; PTH: parathyroid hormone; 25-OH vit D: 25-hydroxy vitamin D

vitamin D and serum PTH levels in the hyperbilirubinaemia group. Additionally, the fact that no difference was determined in Ca, P and ALP levels between the study and control groups have be attributed to the short duration of vitamin D insufficiency or deficiency in the Group 1. The normal vitamin D levels in the mother's corroborate this.

In addition to vitamin D's effect on bone metabolism, it has also been shown to have antiproliferative, pro-differentiative and immunomodulatory effects.[12] Vitamin D has also been reported to have antioxidant properties. Vitamin D3 (cholecalciferol) and its active metabolite 1,25-dihydroxycholecalciferol, as well as vitamin D2 (ergocalciferol) and 7-dehydrocholesterol (pro-vitamin D3) all inhibit iron-dependent liposomal lipid peroxidation.[13] Our literature search revealed no prior studies investigating the relationship between bilirubin metabolism and vitamin D levels. Although there was no difference between the hyperbilirubinaemia and control groups in terms of type of feeding, mothers' ways of dressing and exposure to sunlight, and mothers and babies received no regular vitamin D support, the difference between babies' 25-OH vitamin D levels suggests a correlation between bilirubin levels and vitamin D metabolism at the pathological level. Jaundice is less seen in infants that have exposure to sunlight.[14] This

supports that there may be a relationship between vitamin D and jaundice.

In the first months of life, serum vitamin D levels in newborns correlate with maternal serum vitamin D due to transplacental transfer of vitamin D. However, there was no difference in maternal vitamin D status between the two groups in our study. High PTH levels in the hyperbilirubinaemia group may contribute to low vitamin D by converting 1,25-dihydroxycholecalciferol. Our study is the first on this subject matter. Limitation of this study is that we could not delineate clearly which mechanisms cause vitamin D deficiency in the study group. Further study is needed to explain this situation.

In conclusion, there is an association between indirect hyperbilirubinaemia and serum vitamin D levels in newborns with jaundice at a level necessitating phototherapy. We conclude that our study needs to be verified by future research.

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