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Serum Vitamin D Levels in Term Neonates with Early Onset Sepsis

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Abstract

Background: Neonatal sepsis is one of the commonest causes of neonatal morbidity and mortality. Despite recent advances in risk assessment and prevention for neonatal sepsis, it remains a significant global health burden. Among these, vitamin D deficiency has been proposed as one of the risk factors for neonatal sepsis.

Objective: To measure and compare the vitamin D levels in term neonates with early onset sepsis (case) group and control group.

Methods: Hospital-based case control study conducted between January 1st, 2016 and December 31st, 2016 at Special Care Baby Unit (SCBU) and Maternity Wards I, II and III of Central Women Hospital, Mandalay, Myanmar. Forty term neonates with early onset sepsis and 40 healthy term neonates were included. Cord blood vitamin D levels were measured in both case group and control group.

Results: In early onset sepsis group, all 40 cases of cord blood 25-hydroxyvitamin D levels were deficient (<20 ng/ml). In control group, 26 (65%) cases were deficient (<20 ng/ml) and 1 (2.5 %) were sufficient (≥ 30ng/ml) and 13 (32.5%) were insufficient (20-30 ng/ml). Cord blood 25-hydroxyvitamin D levels of sepsis infants (Mean ± SD of 9.82 ±2.65) were significantly lower than those of the control group (Mean ± SD of 18.47 ±4.37) (p <0.001).

Conclusion: Cord blood 25-hydroxyvitamin D levels of neonates with early onset sepsis were significantly lower than those of the healthy controls. Further studies are needed to establish low level of cord blood vitamin D as a risk factor for EONS.

Keywords: Neonatal sepsis; Serum vitamin D levels; 25-hydroxyvitamin D; Enzyme Linked Immunosorbent Assay; Cord blood vitamin D

Abbreviations: 25(OH)D: 25-Hydroxyvitamin D; AGA: Appropriate for gestational age; CRP: C-Reactive Protein; ELISA: Enzyme Linked Immunosorbent Assay; EONS: Early onset neonatal sepsis; SCBU: Special Care Baby Unit; WHO: World Health Organisation.

Introduction

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. Neonatal sepsis can be classified into two groups: early and late onset sepsis. Early onset neonatal sepsis (EONS) presents within the first 72 hours of life. Neonatal sepsis is a significant cause of mortality and morbidity in newborn babies. Neonatal sepsis accounts for 10% of all neonatal mortality. Neonatal infection is present in 8 of every 1000 live births and 71 of every 1000 neonatal admissions [1]. In the developing world, the neonatal septicaemia remains as a major cause of mortality in spite of recent advances in the technology and therapeutics.

According to WHO data, under-five mortality rate per 1000 live births was 50 and neonatal mortality rate per 1000 live births was 26.4 in Myanmar in 2015. Neonatal mortality was nearly two third of infant mortality. The three major causes of neonatal deaths worldwide are infections (36%), which includes sepsis/pneumonia, tetanus and diarrhea, prematurity (28%) and birth asphyxia (23%) in 2015 [2].

The association between infections and 25-hydroxyvitamin D (25(OH)D) deficiency was first described more than a century ago. Monocytes and macrophages possess 1-α hydroxylase and the active metabolite of vitamin D can also be synthesized in the immune system. The common hypothesis that vitamin D deficiency may play a role in the pathogenesis of infections and low level of circulating vitamin D has been shown to be strongly associated with infectious diseases [3].

There is overwhelming experimental evidence that vitamin D has an important role in the regulation of both the innate and acquired immune systems [4,5]. Newborns are more susceptible to infections as both innate and acquired immune systems are not entirely developed [6]. Therefore, low vitamin D status is expected to be one of the risk factors for early onset neonatal sepsis.

Although 25-OHD can be synthesized by the fetal kidney; neonatal vitamin D levels are primarily dependent on and correlated with the maternal vitamin D status at delivery until the infant starts to receive vitamin D from other sources [7]. Vitamin D deficiency is common in pregnancy and its prevalence ranges from 18 to 84% depending on the country and local clothing customs [8].

Lower vitamin D levels are associated with increased risk of early onset neonatal sepsis in term infants and their mothers. Neonatal and maternal vitamin D levels in sepsis group were significantly lower than those of healthy group [6].

In this study, cord blood vitamin D levels was measured to find out whether there was any association with vitamin D levels and early onset neonatal sepsis in term neonates.

Materials and Methods

The study group consisted of a total of 40 cases of early onset neonatal sepsis (case group) and 40 control babies for cord blood vitamin D level. Sample size was calculated by using statistic software Stata 13 (Confidence interval= 95%, Power of the test = 80%). This study was conducted at Central Women Hospital, Mandalay from January 2016 to December 2016.

Mothers were explained about the procedure in details and informed consents and maternal health questionnaires were taken after taking of cord blood samples at maternity units. History taking and data collection according to pro forma were done.

Immediately after birth, the umbilical cord were clamped and cut, and the baby was handed over to a doctor (or) nurse. About 2 ml of blood from umbilical cord of placental side was collected into a plain sample tube.

After labelling, the cord blood samples were allowed to clot at room temperature and were sent to Common Research Laboratory, University of Medicine, and Mandalay. The samples was centrifuged to separate serum at 2000 rpm for 20 minutes and stored at -20'C until determination was done.

The cord blood samples of maternal risk factors for infections such as prolonged rupture of membrane, intrapartum maternal fever and outside handling were not included in control cases and these were properly discarded according to ward protocol. The cord blood samples of maternal metabolic diseases, diabetes mellitus, thyroid diseases, renal diseases and chronic liver

diseases; babies with gross congenital abnormalities, refusal of parent's consent were not included both control and case group.

Daily assessment of neonates whose cord blood was taken was done up to 3 days of life to differentiate between sepsis group and control group.

Among those samples, only samples of term appropriate for gestational age (AGA) neonates≤ 3 days of age who admitted with clinical diagnosis of early onset sepsis according to SCBU protocol and positive blood culture or positive CRP in any time within 3 days of life were studied for cases.

Healthy term AGA neonates found to be normal up to 3 days of life without clinical signs and symptoms of neonatal sepsis were studied for control group. A collection of information was recorded in pro forma. Randomization was performed by selecting a neonate born within the same week of sepsis case.

Serum 25(OH) vitamin D levels of both case and control groups was determined by 25-OH vitamin D ELISA test attached with Human plus HumaReader (ELISA reader) and microplate washer at Public Health laboratory, Mandalay, Myanmar for comparison.

The data was analyzed statistically with the use of Stata 13 statistical software. The results were shown in frequency distribution tables. Serum vitamin D levels were compared between each group by using Students' test.

Results

The study group consisted of a total of 40 cases of early onset neonatal sepsis and 40 control babies for serum vitamin D levels in term neonates. Out of 80 cases of both case and control groups, 21 (52.5%) cases were male and 19(47.5%) were female in each group. Male: Female ratio was found to be 1.1:1.

In 40 cases of early onset sepsis, 14(35%) cases were between 2.5 and 3kg and 26(65%) were >3kg. Within early onset sepsis cases, 35 (87.5%) cases were found to have association with maternal risk factors where as 5 (12.5%) cases were found not associated with maternal risk factors. In control group, 13(32.5%) were between 2.5 and 3kg and 27(67.5%) were >3kg. Therefore, there was no significant difference between two groups in terms of birth weight and sex. Likewise, there was no significant difference with regard to maternal age, race, birth season and also mode of delivery.

In early onset sepsis group, all 40 cases of serum 25-hydroxyvitamin D levels were deficient (Table 1). In control group, 26 (65%) cases were deficient, 13(32.5%) were insufficient and 1 (2.5%) were sufficient (Table 2). Cord blood 25-hydroxyvitamin D level in case group were significantly lower than that of control group (Mean ± SD of 9.82 ±2.65 and 18.47 ±4.37 respectively; Table 3).

The relationship between vitamin D level and isolation of bacteria pathogen was also assessed. Out of 40 cases of clinically suspected neonatal sepsis, bacteria pathogen was isolated from only one case (2.5%) in this study. This was caused by *Klebsiella pneumoniae*. But there was no significant association between culture positive and vitamin D level in this study. None of the neonates expired during the study period.

Serum 25(OH)D level	Number	Percentage
Deficiency(<20 ng/ml)	40	100%
Insufficiency(20 -30 ng/ml)	0	0
Sufficiency(>30 ng/ml)	0	0
Total	40	100%

Table 1: Cord blood 25-hydroxyvitamin D levels in case group.

Serum 25(OH)D level	Number	Percentage
Deficiency(<20 ng/ml)	26	65%
Insufficiency(20 -30 ng/ml)	13	32.5%
Sufficiency(>30 ng/ml)	1	2.5%
Total	40	100%

Table 2: Cord blood 25-hydroxyvitamin D levels in control group.

	Case	Control	T	P-value
Cord blood 25(OH)D level (Mean ± SD)	9.82 ±2.65	18.47 ±4.37	-10.698	< 0.001

Table 3: The comparison of cord blood 25-hydroxyvitamin D level between case and control groups (Mean ± SD).

Discussion

Neonatal sepsis remains one of significant health care burden despite improvement in risk assessment and prevention. There are many risk factors for EONS like

prematurity and low birth weight, premature or prolonged rupture of membranes, maternal peripartum fever and others.

Therefore, any contribution to understanding of disease process is valuable. Moreover, the association between neonatal sepsis and 25-hydroxyvitamin D status has attracted attention in recent year. Low vitamin D status is one of the risk factors for early onset neonatal sepsis. Cetinkaya, et al. [6] and Cizmeci, et al. [3] showed that vitamin D levels of neonates with EONS were significantly lower than those of healthy controls.

Vitamin D deficiency is associated with several adverse health outcomes. Vitamin D has an emerging role in regulating inflammation as well as an important role in immunomodulation. Therefore, the impact of vitamin D on immune system has been extensively reviewed in recent years. Moreover, vitamin D deficiency has been associated with increased sepsis in children and adults stated by Kate Madden [10].

In Myanmar, there are few studies on serum vitamin D level in adults, for example, serum vitamin D levels in patients with stable chronic obstructive pulmonary disease by Ni-Ni-Win-Swe [11]. Also in 2015, the study of serum vitamin D level and disease activity in patients with systemic lupus erythematosus was done by Aye Thin Zar Oo [12]. There is no study on serum vitamin D level in neonates in Myanmar.

The present study was aimed to find and compare cord blood vitamin D levels in term neonates between sepsis group and control group. Forty cases of early onset sepsis and forty cases of healthy control neonates from Central Women Hospital, Mandalay, Myanmar were included in this study within one year period according to inclusion and exclusion criteria.

Out of 80 cases of both case and control groups in this study, 21 (52.5%) cases were male and 19(47.5%) were female in each group. From the study of Cizmeci (2014) in Turkey, 27(68%) were male and 13(32%) were female in case group and 28(65%) and 15(35%) were male and female respectively. These results may be due to the fact that males are more affected than females in neonatal sepsis and may also due to small sample sizes.

Birth weight of Mean ± SD in grams was 3075 ±445 in case group and 3445 ±385 in control group in this study. Similarly, Cizmeci [3] in Turkey stated that birth weight of Mean ± SD in grams was 2877 ±652 in case group and 3120 ±440 in control group.

In a study done by Mirzaei, et al. [9] stated that vitamin D deficiency was higher among small for gestational age newborns in comparison to appropriate for gestational age newborns (25% vs. 17.5%). Therefore, only term appropriate for gestational age neonates were selected and small for gestational age neonates were not included in this study due to risk of cofounding factor.

Out of 40 cases of clinically suspected neonatal sepsis, bacteria pathogen was isolated from only one case (2.5%) in this study. This was caused by *Klebsiella pneumoniae*. But there was no significant association between culture positive and vitamin D level in this study.

Although blood culture results are important for diagnosing neonatal sepsis, it has a low rate of culture in this study as the laboratory attached to the hospital was not equipped in standard level and not getting the result within a week. It was difficult to diagnose neonatal sepsis early and resulting in unnecessary or delayed treatment. Culture negative sepsis cases were included in the study, when the babies were met diagnostic criteria of early onset sepsis.

Among the clinically suspected early onset sepsis, all cases are qualitative CRP positive in any time within 3 days of life in this study.

In this study, all 40 cases of cord blood 25-hydroxyvitamin D levels were deficient (<20 ng/ml) in early onset sepsis group. 26 (65%) cases were deficient (<20 ng/ml), 13(32.5%) were insufficient (20-30 ng/ml) and 1 (2.5%) were sufficient (>30 ng/ml) in control group.

Vitamin D status varies in different countries due to differences in exposure to sunlight, dietary intake of vitamin D, ethnicities and cultural factors. Vitamin D level is expected to be normal in Myanmar due to adequate sunshine area.

However, in this study, only one case was sufficient (>30 ng/ml) and remaining 39 cases in healthy group are not sufficient (< 30 ng/ml) in control group and all cases in sepsis group are deficient (<20 ng/ml) which may be due to nutritional deficiency of vitamin D in mothers during pregnancy and partly also due to small sample sizes.

Similarly, according to the study of Cizmeci [3] in Turkey, 5(12.5%) were sufficient (≥ 30 ng/ml) and 35(87.5%) were low (<30ng/ml) in case group. Among the babies with low levels of cord blood 25-

hydroxyvitamin D, 28(70%) were deficient and 7(17.5%) were insufficient. 20(46.5%) were sufficient (≥ 30 ng/ml), 23(53.5%) were low (<30ng/ml) in control group. Among the babies with low levels of cord blood 25-hydroxyvitamin D, 22(51.2%) were deficient and 1(2.3%) were insufficient.

Another study from Turkey by Cetinkaya [6] stated that 42(84%) were <11 ng/ml and 8(16%) were 11-32 ng/ml in case group and 1(2%) were <11 ng/ml and 49(98%) were 11-32 ng/ml in control group. It was comparable to this study.

Moreover, this study showed that cord blood 25-hydroxyvitamin D levels in case group were statistically significant lower than that of control group (Mean ± SD of 9.82 ±2.65 and 18.47 ±4.37 respectively), (p-value <0.001). Low levels of cord blood 25-hydroxyvitamin D levels were associated with increased risks of EONS.

It was comparable to the study in Turkey by Cetinkaya [6] who also stated that both maternal and neonatal 25-OHD levels in the study group were significantly lower compared with those in the control group.

Moreover, a study done by Xue Tao [13] in China showed that cord blood CRP was inversely associated with vitamin D in neonates with low 25-hydroxyvitamin D level. That fact also supports that vitamin D deficiency is associated with infection, inflammation and sepsis.

In this study, there is strong association between early onset neonatal sepsis (EONS) and vitamin D level. Besides, some authors [3,6] suggested that adequate vitamin D supplementation during pregnancy is recommended to reduce EONS. Therefore, continued research will help us better understand the immunomodulatory role of vitamin D and the effect of early vitamin D supplementation in mothers on EONS.

Based on currently available data, there is no enough evidence to recommend for treatment with vitamin D in neonates according to cord blood vitamin D level and also measuring cord blood vitamin D level to predict EONS is still investigating. In conclusion, there are many different measures to reduce EONS; measurement of cord blood vitamin D level may possibly become one of these measures.

Limitations

There were limitations in this study. Firstly, lack of measurement of maternal serum 25-hydroxyvitamin D level in this study is one limitation. Therefore, it cannot prove lower cord blood 25-hydroxyvitamin D level may be due to lower maternal serum 25-hydroxyvitamin D level in both sepsis and control groups. Secondly, a small sample size may be limitation of this study. The last limitation may be lack of normal values of serum 25-hydroxyvitamin D level in both maternal and neonatal population of Myanmar.

Conclusions

In the present study, forty cases of early onset sepsis and forty cases of healthy control neonates are studied to find and compare cord blood vitamin D level in both groups. Cord blood 25-hydroxyvitamin D levels in case group were statistically significant lower than that of control group (Mean ± SD of 9.82 ± 2.65 and 18.47 ± 4.37 respectively), (p-value <0.001).

It is hoped that adequate vitamin D supplementation to mothers may play a role in the prevention of EONS in the future.

Moreover, 65% of cases are also deficient(<20 ng/ml) in control group in this study that may be lack of data in local vitamin D levels of neonates in Myanmar, or due to nutritional deficiency of vitamin D in mothers during pregnancy. It is suggested that further larger studies are required to determine the ideal range of maternal and neonatal serum 25-hydroxyvitamin D levels.

Supplemental Information

Clinical Diagnosis of Early Onset Neonatal Sepsis (SCBU, protocol)

Clinical diagnosis of early onset neonatal sepsis is defined asin the presence of any one or more of the following clinical signs and symptoms. They are temperature instability(hypothermia (<96°F / <36°C), fever (>100°F / >37.8°C)), lethargy or irritability, skin (unexplained cyanosis or jaundice, pallor, skin mottling and rashes), feeding problems (reduced feeding or unable to suck, vomiting, diarrhea and abdominal distension), Cardio-pulmonary (increased respiratory rate >60/min, respiratory distress (grunting, nasal flaring and retraction), apnoea, poor perfusion (prolonged capillary refill time> 3 sec)), central nervous system (convulsion, hypotonia, hypertonia, abnormal movement, bulging fontanelle, impaired neonatal reflexes) and focal infection (impetigo, cellulitis, soft tissue abscess, umbilical sepsis and conjunctivitis).

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