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Association of vitamin D plasma concentration with the severity of illness among children with sepsis treated in Pediatric Intensive Care Unit

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Abstract

***Objective*: To investigate whether vitamin D plasma concentration correlated with the severity of illness in sepsis children treated in a Pediatric Intensive Care Unit (PICU).**

***Design*: This was a cross sectional study.**

***Settings*: Pediatric Intensive Care Unit of Sanglah Hospital Denpasar, Bali, in May to November 2016.**

***Patients and participants*: Samples were patients aged 28 days to 12 years who had sepsis or severe sepsis or septic shock and have been hospitalized in PICU. The subjects who met the inclusion criteria were divided into two groups based on the vitamin D status: normal and insufficient.**

***Intervention*: The severity of illness of the patients in each group was measured using Pediatric Logistic Organ Dysfunction (PELOD) II and Pediatric Risk of Mortality (PRISM) III score. The demographic data, anthropometric status, and severity of the illness were taken from the medical records. The amount of sun exposure and patient nutritional intake were taken from questionnaires answered by the parents.**

***Results*: A total of 48 patients were examined in this study. Bivariate analysis showed that vitamin D insufficiency was associated with a higher severity of sepsis based on the PRISM III (r=-0.44, p=0.006) and PELOD score (r=-0.5, p<0.001).**

***Conclusion*: Vitamin D plasma concentration was negatively correlated with the illness severity in children with sepsis.**

Key words: Vitamin D, illness severity, sepsis, critically ill children.

Introduction

Vitamin D along with parathyroid hormone has an important role in metabolism. (1) Some of the latest studies suggested vitamin D has potential benefits as an anti-inflammatory and in the regulation of the immune system. (1,2) Vitamin D supplementation influences the outcome of a patient with a critical or an acute illness. (3) Many studies found macrophages produce 1,25-dihydroxyvitamin D3 or 1,25(OH)2D3. (1,4) Vitamin D receptor (VDR) is expressed in the nucleus of various immune cells, such as CD4+, CD8+, T cells, B cells, neutrophils, antigen-presenting cells (APC), and dendritic cells. (1,4) Specifically, the interaction of 1,25(OH)2D-VDR will trigger a phagocytic process, chemotaxis, cell proliferation, and production of immunoglobulin B. (4-7)

The primary source of vitamin D is converted into 7-dehydrocholesterol to form previtamin D3 in the skin with the help of ultraviolet B radiation. Inactive precursor form must be metabolized by vitamin D-25 hydroxylase in the liver. (5,8) The second process of hydroxylation by cytochrome P450 monooxygenase 25(OH)D 1α happens in the proximal renal tubules. (9) The active form of the final product is called 1,25-hydroxyvitamin D3 or 1,25(OH)2D or calcitriol, which its half-life is only 4 to 24 hours. (1,3) In contrast to its active form, 25-hydroxyvitamin D or 25(OH)D3 or calcidiol has a longer half-life, about 15 days. Because 25(OH)D3 is the most common form of vitamin D measured many studies, it is used to describe a person's vitamin D status. (5,8)

Recent studies demonstrated that low concentration

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of vitamin D in the plasma were prevalent in critically ill adult patients treated in an intensive care unit. (1,3,9) Moreover, vitamin D hypovitaminosis is associated with more severe disease at the beginning of treatment, higher mortality, a poorer short-term outcome, and a long-term worsening in adult intensive care unit patients. (9) McNally et al reported that vitamin D deficiency was associated with a greater severity of disease in critically ill children. (5) Braun et al also claimed vitamin D insufficiency increases the risk of all-cause mortality at day 30 and 36. In addition, a study conducted by Madden et al involving 51 patients in a Pediatric Intensive Care Unit (PICU) showed the level of vitamin D was lower in pediatric patients who were treated in PICU. (10) Moreover, there was a significant association between vitamin D deficiency with the incidence of septic shock. (10)

In infant population, low blood level of vitamin D was more frequently found in patients with early onset of neonatal sepsis. Another study also reported an association between vitamin D plasma concentration of maternal and the infant. (11) However, a study by Lucidarme et al in 2017 in 107 PICU patients obtained no association between vitamin D deficiency and the degree of severity of the disease and sepsis. (11) The finding was supported by a study by Rippel et al which found no association between vitamin D deficiency and the treatment duration or continuity in PICU. (2) A study by Rey et al found vitamin D deficiency could not predict mortality patients in PICU, although this study also showed a high incidence of vitamin D deficiency. (9)

Although several studies have provided information about vitamin D deficiency and its effects in illnesses, the effect in patients with acute critical illness remains unclear. Moreover, there is lack of readily available data on vitamin D deficiency in critically ill children in Indonesia. A comparative study in 2015 conducted by Utami in Hasan Sadikin Hospital, Indonesia, concluded that most children who had been hospitalized had a low level of vitamin D. (12) The study also found a significant difference in vitamin D level between children with critical illness and those without critical illness. (12) The study showed a strong correlation between vitamin D deficiency and critically ill children. (12)

The primary purpose of this study was to determine the correlation of vitamin D plasma concentration with the severity of the disease in children with sepsis in a PICU.

Methods

This study was a cross sectional study. The subjects were all children with sepsis hospitalized in Paediatric Intensive Care Unit (PICU), Sanglah Hospital, Denpasar, Bali. The target population were children over the age of 28 days to 12 years with sepsis who were treated in PICU. The accessible population in this study were children over the age of 28 days to 12 years with sepsis who were treated in PICU of Sanglah Hospital and met the inclusion criteria during the period in 2016.

The inclusion criteria were: (1) age <12 years, (2) the approximate length of stay in PICU ≥48 hours. The exclusion criteria were: (1) the patient did not meet the criteria for sepsis or severe sepsis or septic shock, (2) the patient had any of these conditions: chronic kidney disease, gastrointestinal malabsorption, a state of post-surgery, trauma, autoimmune diseases, immunocompromised state, or malignancy, (3) the patient or the parent or parents refused to enroll in the research.

The first day of the study was the first day a patient met the criteria for sepsis or severe sepsis or septic shock. The parents or guardians were given an informed consent.

We used the Pediatric Logistic Organ Dysfunction (PELOD) 2 score to assess the illness severity. The PELOD 2 score was calculated on the first day and on a daily basis to measure the clinical worsening. The severity of a patient's illness in PICU was measured using Pediatric Risk of Mortality (PRISM) III score. The PRISM III score was calculated in the first 24 hours of treatment.

Each patient had his or her blood sample in 3 ml serum which taken on the first day of the study to assess the level of vitamin D. Each patient's level of vitamin D was measured once by evaluating the 25(OH)D plasma using liquid chromatography in PT Prodia Widyahusada Tbk laboratory, Denpasar, Bali. Each 3 ml of serum separated in aliquot and kept in freezer with -20 °C until all sample were collected. We grouped the patients based on their vitamin D status: sufficient (≥30 ng/mL), vitamin D insufficiency (20-30 ng/mL), and vitamin D deficiency (<20 ng/mL). For the primary outcome, we made two groups: normal 25(OH)D level (≥ 30 ng/mL) and insufficiency (<30 ng/mL).

The patients' medical records were reviewed to obtain the demographic data, including age, weight, height, gender, date of entry into care, diagnosis, and a history of chronic disease.

The daily intake and the amount of sun exposure of each patient were obtained through a questionnaire given to and answered by the parents. The questionnaire for parents with children older than one year asked about the amount of milk, juice, fortified cereal, and supplemental vitamin D consumption. For

parents of children aged <1 year, the questions are about the children feeding habits. Anthropometric data determined the nutritional status. We use z-score weight/height to subject less than five years, and for the subject more than five years we use Waterlow classification. Nutritional therapy was determined according to nutrients intake option. Nutritional therapy was divided into parenteral and enteral nutrition; and timing of nutrients that was split into two categories namely more than 48 hours since being treated, or before 48 hours since treated. More clinical information was collected during the admission and discharge, including the length of stay in PICU, the duration of ventilator use, the duration of shock, the PELOD score, and the mortality. The subjects were followed prospectively to determine the duration of treatment in PICU by recording the patient admission to PICU and the discharge date. We also recorded whether the patient used a mechanical ventilation, the duration of antibiotic usage calculated based on the number of days the patient received antibiotics, the nutritional therapy as described previously, and the patient outcome (mortality).

The number of subjects required was calculated using a formula for a correlation study. (13) Based on the calculation, our study required a minimum number of 48 subjects.

The demographic and clinical data were analyzed using Shapiro-Wilk. The data were reported in the form of continuous mean and standard deviation (SD) for the normally distributed. We used the median and interquartile range for the skewed distributed data. The categorical data was displayed in the form of proportion (%).

Statistical analysis was performed by computer to determine the correlation between the plasma concentration of 25(OH)D and the outcome such as the severity of the disease. A p value less than 0.05 was considered significant.

Results and discussion

Participants

The study was done for seven months. During that study period, 245 children with critical illness had been hospitalized in PICU. Only 52 children were presenting sepsis. One subject refused to join our study, two subjects were with malignancy, and one subject had a secondary immunodeficiency. Eventually, 48 subjects were enrolled and analyzed (**Figure 1**).

Subject characteristics

From 48 subjects, 28 were males (58.3%) and 20 females (42.7%). A similar study by Dewi also found more males had sepsis (64%) than females. (14) Another study by Saraswati found a similar result that there were more males (55.4%) in a group of patients with sepsis. (15)

In our study, 29 subjects (60.4%) were 1- to 12-month-old. This characteristic is similar to a study by Saraswati, where most sepsis patients aged 1- to 12-month-old (62%) and the sepsis morbidity decreased as the age rose. (15) A younger age correlates with immune system immaturity. Therefore, a younger child will be more susceptible to sepsis than an older one.

Based on the nutritional status, 70.8% of our subjects was with a good nutritional status. The study by Saraswati, which 57.6% of the sample had a poor nutritional status, stated that the children with a poor nutritional status were the most susceptible to sepsis. (15)

In this study, we found the mean of vitamin D level among children with sepsis was 16.18 ng/ml. Another study conducted by Utami in Indonesia involving critically ill pediatric patients found the mean of vitamin D among critical patient was 12.6 ng/ml. (12) A study in the US by Madden found the mean of vitamin D was 22.5 ng/ml. (10) A developed country may have children with higher vitamin D plasma concentration as a result of supplementation of multivitamins or vitamin D on a regular basis. In contrast, none of our subjects had a good intake of multivitamin or vitamin D supplementation. The study by Utami also showed that neither the critically ill subjects nor the non-critically ill had been given multivitamin or vitamin D. A study conducted in the US by Kumar found only 4% of the subjects regularly take vitamin D supplementation. (16) In our study, we found 37 subjects (77.1%) had vitamin D deficiency, 11 (22.9%) had vitamin D insufficiency, and no one had sufficient level of vitamin D. A study by Utami found 88% of the critically ill patients had vitamin D deficiency, 12% had vitamin D insufficiency, and no one had a sufficient level of vitamin D. (12) A study in the US by Jeng showed the prevalence of vitamin D deficiency in critically ill patient with sepsis was 100%. (17)

Table 1 shows the subject characteristics in this study. According to Shapiro-Wilk normality test, the vitamin D level and the PRISM III score are normally distributed. However, the age, length of stay, and PELOD score are not normally distributed.

Correlation between the level of vitamin D and the degree of sepsis severity in children

In bivariate analysis, we found a significant moderate negative correlation between vitamin D

plasma concentration and the severity of illness in children with sepsis as assessed by PRISM III score (r=-0.44, p=0.006). We also found a significant moderate negative correlation between vitamin D concentration and the severity of illness in children with sepsis as assessed by PELOD score (r=-0,5, p<0,001) (**Table 2**). A study by Madden found a similar significant negative correlation between vitamin D plasma concentration and the severity of sepsis in children when assessed using the PRISM III score (r=-0.45, p<0.001). (10) A study by Ebenzar found a weak negative correlation between a low vitamin D plasma concentration and a higher severity illness as measured by Pediatric Index of Mortality (PIM) 2 score (r=-0.29, p=0.04). Our study did not use this score because we had several limitation to predict mortality in PICU and needed further recalibration. (18,19) The study found no significant correlation between vitamin D plasma concentrations with the length of stay. (18)

Patient with severe infection like sepsis had a high prevalence of severe vitamin D deficiency and a high mortality. Several studies asserted vitamin D insufficiency as a risk factor of sepsis. The role of vitamin D therapy in sepsis had been studied in mice with sepsis showed that in sepsis accompanied with dissemination of intravascular coagulation, 1,25(OH)2D3 administration increased the parameters of coagulation. Vitamin D administration has been studied in vitro. The studies showed that vitamin D not only regulated the release of cytokine inflammatory agents like TNF-α and IL-6, but also inhibited lipopolysaccharide (LPS) activation. LPS is an important molecule that plays a role in sepsis caused by gram-negative bacteria and in vascular endothelial vasodilation. Vitamin D function in inhibiting sepsis cascade is a key role of vitamin D in sepsis mechanism. In the cascade of sepsis caused by a fungal infection, a study by Khoo found that vitamin D therapy in Candida albicans infection caused a significant reduction of pro-inflammatory cytokines production induced by the fungi. (19)

Our study only found a moderate negative correlation between vitamin D level and the severity of sepsis in pediatric patients (**Figures 2** and **3**). The mean PELOD 2 score was 13. The mean PRISM III score was 15.94. The scores showed that our subjects had high severity index. The study used 2 parameters (PELOD and PRISM) to evaluate severity of illness because severity of illness might be proven in the first 48 hours when the blood sample was taken. The results showed only moderate negative correlation, which we suspected that was due to the study not divided insufficiency for analysis further. Study by Utami S et al in Indonesia, divided insufficiency to deficiency and insufficiency, therefore the association of vitamin D and critically ill patient is higher. A study by Madden found a similar significant negative correlation between vitamin D plasma concentration and the severity of sepsis in children when assessed using PRISM III score (r=-0.45, p<0.001). Level of vitamin D in Madden study was also more higher (22.5 [16.4-31.3] ng/mL) than this study (16.18±6.46 ng/mL). The different result was probably caused by small sample size in our study than Madden study. And the variability of subject can influence the result of the study with small sample size.

Therefore, further study with large sample size and identification of deficiency vitamin D status in association with critically ill patients is needed.

Our study cannot determine the primary aetiology of the disease in each subject in PICU. Tertiary hospitals such as ours had some difficulties in determining the single primary cause of sepsis in paediatric patients because most of the patients were referred with several diagnosis and complications.

Conclusions

In bivariate analysis, a significant moderate negative correlation was found between vitamin D levels and the severity of illness in children with sepsis as assessed with PRISM III score (r=-0.44, p=0.006) and the PELOD 2 score (r=0.5, p<0.001).

Study limitation

Our study only had a small sample size. Further studies are needed to evaluate the association between the aetiology of sepsis or organ involvement and vitamin D deficiency, preferably with a larger sample size.

Conflict of interest

The authors declare there was no conflict of interest in conducting the study and writing the article.

Table 1. Subject characteristics

Characteristics	Sample (n=48) f (%)	Mean±SD or Median (IQR)
Sex - Male	 28 (58.3)	
Age (months) - 1-12 - 13-60 - 61-144	 29 (60.4) 10 (20.8) 9 (18.8)	8.5 (37)
Nutritional status - Low - Good - High - Obesity	 8 (16.7) 34 (70.8) 4 (8.3) 2 (4.2)	
History of breastfeeding - Yes	 39 (81.2)	
Formula intake - Yes	 19 (39.6)	
Administration of multivitamin/vitamin D - No	 48 (100)	
Level of vitamin D (ng/mL)		16.18±6.46
PELOD 2 score		31.5 (13)
PRISM III score		15.94±6.46
Mechanic ventilation use - Yes	 45 (93.8)	
Length of stay (days)		7 (6)
Outcome - Death	 37 (77.1)	

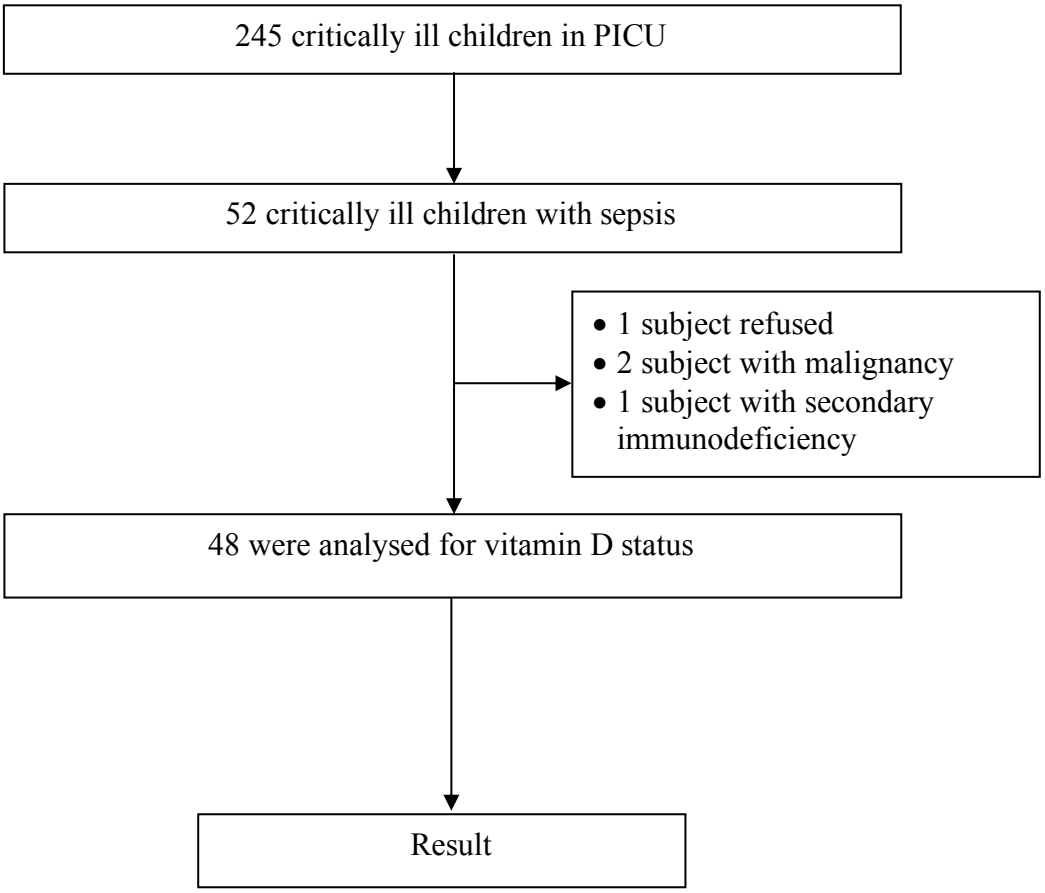
Legend: PELOD 2=Pediatric Logistic Organ Dysfunction 2; PRISM III=Pediatric Risk of Mortality III.

Table 2. Correlation of vitamin D plasma concentration to the severity of sepsis

Vitamin D plasma concentration correlation to	r*	p
- PRISM III score	-0.44	0.002
- PELOD 2 score	-0.5	0.000

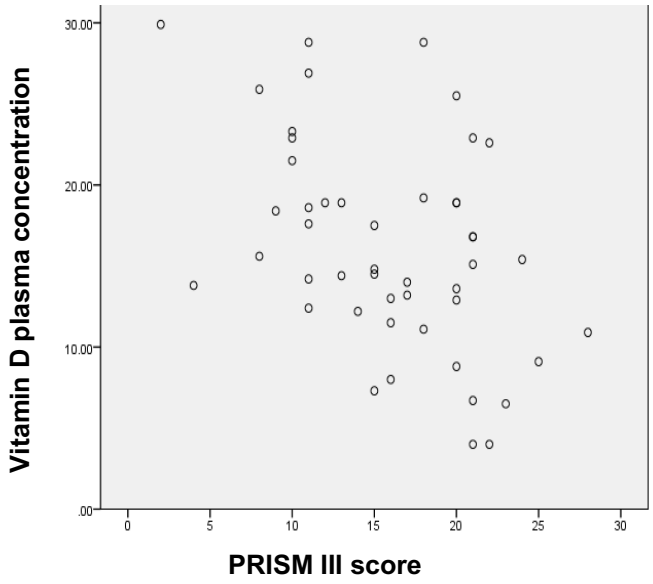
Legend: *=correlation by Pearson analysis; PRISM III=Pediatric Risk of Mortality III; PELOD 2=Pediatric Logistic Organ Dysfunction 2.

Figure 1. The flow diagram of the study participants



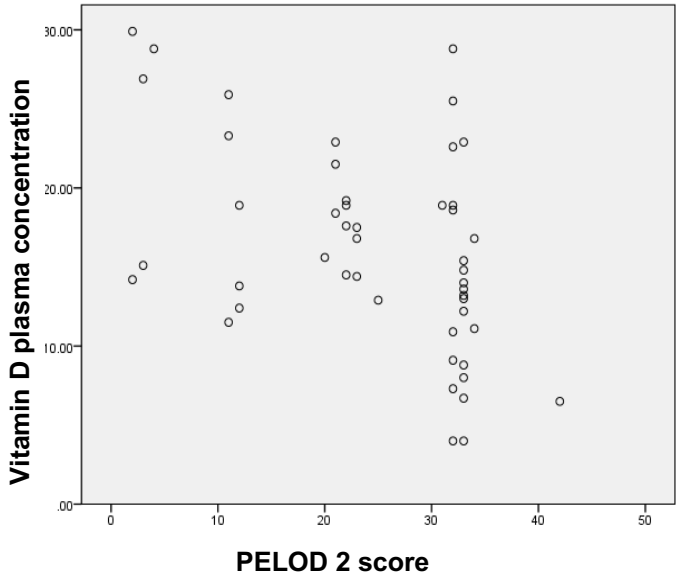
Legend: PICU=Pediatric Intensive Care Unit.

Figure 2. A scattered plot showing vitamin D plasma concentration correlation to the severity of sepsis based on PRISM III score



Legend: PRISM III=Pediatric Risk of Mortality III.

Figure 3. A scattered plot showing vitamin D plasma concentration correlation to the severity of sepsis based on PELOD 2 score



Legend: PELOD 2=Pediatric Logistic Organ Dysfunction 2.

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