Vitamin D deficiency and outcome of patients with sepsis in pediatric intensive care unit: a prospective observational study

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Abstract

***Background*: Vitamin D is important in immunomodulation, regulation of inflammation and cytokines, cell proliferation, cell differentiation, apoptosis, angiogenesis, muscle strength, and muscle contraction. Patients with sepsis have high mortality rate and high deficiency in vitamin D. (1) Vitamin D is increasingly recognized as an important mediator of immune function and may have a preventive role in the pathogenesis of sepsis. (2) Vitamin D also influence cardiovascular function. (3) We aimed to find the correlation of vitamin D level with severity of sepsis in patients admitted to pediatric intensive care unit (PICU).**

***Setting*: Participants and laboratory were collected from patients with sepsis who admitted to the PICU at Dr. Sardjito General Hospital, Yogyakarta.**

***Patients and Participants*: Participants pediatric sepsis patients admitted to the PICU from September 2015 to April 2016. Blood samples for 25-hydroxyvitamin D [25(OH)D] concentration were collected at the first 24 hours admission in PICU using ELISA method. Other data recorded include pediatric logistic organ dysfunction (PELOD) score at admission, PICU length of stay and mortality.**

***Measurement and results*: A total of 297 patients were admitted to the PICU during the 8-month study period. Fourty-two patients had diagnosis of sepsis, severe sepsis or septic shock on PICU admission. Of all these studied patients, 25(OH)D deficiency was identified in 23 (54.8%) patients, insufficiency in 9 (21.4%) patients, and normal levels in 10 (23.8%) patients. PICU mortality was higher in patients with 25(OH)D deficiency, ie 7 (30.44%) patients, but it did not show any statistical significance compared to the group of patients with 25(OH)D insufficiency or normal level of 25(OH)D group of patients (p=0.78). The group with normal level of 25(OH)D had the highest PELOD score but on the other hand they have the lowest mortality rate.**

***Conclusions*: There is a high prevalence of vitamin D deficiency in pediatric sepsis patients admitted to PICU, but not correlated with PELOD score on admission. PICU lenght of stay also not associated with mortality in PICU.**

Key words: Vitamin D, vitamin D deficiency, sepsis, PICU, mortality, lenght of stay.

Introduction

Vitamin D has been increasingly implicated in the proper functioning of multiple organs. Deficiency states associated with cardiovascular disease, asthma, multiple sclerosis, diabetes, acute lower respiratory infection, and cancer. (4,5)

Vitamin D synthesis is initiated in the skin by ultraviolet B (UVB) radiation from the sun activating its precursor 7-dehydrocholesterol, which then circulates in the blood to liver, where it is converted into its main metabolite 25(OH)D, which has blood level about 1000 times higher than the active metabolite, 1,25 dihydroxyvitamin D [1,25(OH)2D]. (6)

The effect of vitamin D on the immune system has been extensively studied. In general, 1,25(OH)2D, the active form from vitamin D, acts not only to promote the innate immune response to microbial pathogens, but also to eradicate what might be an overzealous adaptive immune response to pathogens that prove difficult for the macrophage to handle effectively. (7,8) Although 1,25(OH)2D has direct effects on the adaptive immune system, it also affects the ability of the innate immune system to instruct the adaptive immune response. (9,10) In this instance, 1,25(OH)2D is a potent supressor of IL-12 production. (11)

Recently, vitamin D deficiency has been asscociated with higher illness severity upon admission,

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mortality and worsen short and long term outcomes in adult intensive care unit (ICU) patients. (12,13) Several studies provided new information regarding the relationship between vitamin D status and critical illness in children admitted to pediatric ICUs (PICUs). It was observed that hypovitaminosis D is a common finding in critically ill children. (14) Rippel et al did not find an asscociation between hypovitaminosis D and length of stay or hospital survivor. (15) Rey et al reported high incidence of hypovitaminosis D in PICU and associated with higher prediction of risk mortality scores. (12)

The role of vitamin D in sepsis syndrome has not been fully evaluated in human. We performed a cross-sectional study of vitamin D status and its relationship with outcome of sepsis patiens in PICU.

Materials and methods

Study design and setting

This was a prospective study of all patients with sepsis admitted to PICU between September 2015 and April 2016. Our PICU is an 11-bed unit, university-affiliated hospital, staffed daily by 3 pediatric intensivist and pediatric residents.

Methods

All patients admitted to the PICU with additional diagnosis sepsis were included in the study. Patients readmitted to PICU during the same period of hospital were excluded. 25(OH)2D levels were collected during the first 24 hours of admission to the intensive care unit.

Measurement of serum 25(OH)D: two ml of venous blood were obtained centrifuged and serum was separated and stored at -20^{o}C until assayed. Serum level of 25(OH)D was analyzed automatically using ELISA method supported by SMC (Sensotronic Memorized Calibration) Technology. The device is Alegria® Analyzer, ORGENTEC Diagnostica GmBh, Mainz, Germany. Baseline demographics were age, gender and nutritional state. Patients who have history of kidney disease or other chronic disease were excluded. The PELOD score was collected within 24 hours of PICU admission. PELOD represents the amount of organs dysfunction of the patients at admission. Clinical and laboratory variables obtained during the first 24 hours of PICU admission include serum levels of total calcium, sodium, potassium, creatinine, glucose, albumin and 25(OH)D. Utilization and duration of invasive mechanical ventilation, PICU length of stay (LOS), and PICU mortality were analyzed. LOS in the PICU was defined as from PICU admission to the time of transfer out of the PICU. This study was approved by the institutional research review board of Universitas Gadjah Mada and informed consent was needed.

Definition of vitamin D deficiency

According to Endocrine Society, the normal serum level of 25(OH)D being defined as >30 ng/dL, insufficiency was defined as 20 to 29.9 ng/dL and the level <20 ng/dL was defined as deficiency. (16) Studies examining 25(OH)D in intensive care units have no agreement on the cut-off level for critically ill patients, with deficiency being defined as 25(OH)D levels of less than 10 ng/dL to less than 29 ng/dL. In our study, we used a 25(OH)D cut-off level of less than 20 ng/dL to define 25(OH)D deficiency, and 25(OH)D insufficiency was defined as 20 to 29.9 ng/dL.

Definition of sepsis

Sepsis was defined as SIRS associated with infection. The presence of at least two of four criteria: temperature, leukocyte, tachypnea and tachycardia. One of which must be abnormal temperature or leukocyte count. Core temperature of >38.5°C or <36°C. Tachycardia, defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli or otherwise unexplained persistent elevation over a 0.5- to 4-hr time period, or for children <1-year-old. Bradycardia, defined as a mean heart rate <10th percentile for age in the absence of external vagal stimulus, β-blocker drugs, or congenital heart disease; or otherwise unexplained persistent depression over a 0.5-hr time period. Mean respiratory rate >2 SD above normal for age or mechanical ventilation for an acute process not related to underlying neuromuscular disease or the receipt of general anesthesia. Leukocyte count elevated or depressed for age (not secondary to chemotherapy-induced leukopenia) or >10% immature neutrophils. (17)

Infection defined as suspected or proven (by positive culture, tissue stain, or polymerase chain reaction test) infection caused by any pathogen or a clinical syndrome associated with a high probability of infection. (18) Evidence of infection includes positive findings on clinical examination, imaging, or laboratory tests (white blood cells in a normally sterile body fluid, perforated viscus, chest radiograph consistent with pneumonia, petechial or purpuric rash, or purpura fulminans). (8) Sepsis defined as SIRS in the presence of or as a result of suspected or proven infection. Severe sepsis defined as sepsis plus one of the following: cardio-

vascular organ dysfunction or acute respiratory distress syndrome or two or more other organ dysfunctions. (19)

Statistical analysis

Data analysis was conducted using the SPSS v21.0. Discrete variables are expressed as counts (percentage) and continuous variables as means± standard deviations (SD). For the demographic and clinical characteristic of the patients, differences between groups were assessed using the chi-squared test and Fisher's exact test for categorical variables and the student's t-test or Mann-Whitney U test for continuous variables. A one-way analysis of variance (ANOVA) was performed to explore the impact of admitting diagnosis classified base on source of infection in five different categories: (a) neurologic, (b) pulmonary, (c) gastrointestinal, (d) urinary tract infection, and (e) sepsis on the continous dependent variable outcome of vitamin D levels. A multiple logistic regression model was performed for the whole population with mortality as the dependent variable and age, gender, PELOD score, ventilator requirement.

Results

Baseline characteristics

A total of 297 patients were admitted to the PICU during the 8-month study period. Of these, 42 patients had diagnosis of sepsis, severe sepsis or septic shock at PICU admission. 25(OH)D levels were available for 42 (14.14%) patients. Of all these studied patients, 25(OH)D deficiency was identified in 23 (54.8%) patients, insufficiency in 9 (21.4%) patients, and normal levels in 10 (23.8%) patients (**Figure 1**). Characteristics of the patients were stratified according to 25(OH)D levels on admission (**Table 1**). There were no difference in the mean 25(OH)D levels among age, sex, nutritional state, ventilator requirement for the first 48 hours after admission and initial procalcitonin level. Comparisons of 25(OH)D level among different admission state of sepsis grup and different underlying infections did not show statistical significance (**Table 2**).

Outcome

PICU mortality was higher in patients with 25(OH)D deficiency ie 7 (30.44%) patients, but it did not show any statistical significance compared to the group of patients with 25(OH)D insufficiency or normal level of 25(OH)D group of patients (p=0.78). The group with normal level of 25(OH)D had the highest PELOD score, but on the other hand they have the lowest mortality rate (**Table 3**). There were no difference in PICU LOS among groups.

Disccusion

The study demonstrated that in a sample of critically ill children with sepsis, severe sepsis or septic shock admitted to PICU, the prevalence of hypovitaminosis D was high. This result supports recent investigation showing that hypovitaminosis D is common in critically ill children. (15,20) It was observed that 54.8% of the present PICU patients had 25(OH)D<20 ng/mL, higher to the rate of 29.5% from the study by Rey et al in north of Spain, the rate of 34.5% from Rippel et al in a cohort of critically ill Australian children, and also higher than the 40.1% and 69% reported by McNally et al from North American and Canadian children. (21)

The 25(OH)D levels from the presents PICU patients were compared with the 25(OH)D levels that were obtained as part of a study on vitamin D difference in nutrient biomarkers concentration by habitual intake of milk among preschool children in an urban area of Indonesia that were also done in Yogyakarta. The prevalence of vitamin D deficeincy in Yogyakarta population of healthy children was 63.5% that was higher to the reported prevalence of 15.6% in Rey et al study from North of Spain healthy children population and also 18% in Mansbach's population-based study of north American children, but similiar with the published prevalence of vitamin D deficiency in North American and Australian adolescents ranged from 29%-68%. (12) The probable reason for high prevalence of deficiency in our population is that the quality of daily dietary intake in healthy children in our setting. Vitamin D could be obtained from food, but very few foods naturally contain vitamin D (such as liver, beef, veal, eggs and salmon and in our setting consumption of these foods are low. (7,21) Beside foods, the most important source of vitamin D is from its synthesis in the skin upon exposure to UVB radiation. (22,23) In tropical countries which close to equator line, such as Indonesia, UVB ray is the most intense allowing vitamin D synthesis in the skin throughout the year. Therefore, high prevalence of vitamin D deficiency found in our setting in healthy children population seems unbelievable, but other studies also have shown high vitamin D deficiency in countries with high exposure to sunlight. It have been shown that less time spent outdoors as well as low social economic situation, low milk consumption, vegetarian diet, limitation supplement use and low fish intakes are risk factors of vitamin D deficiency. (24) It cor-

related well between prevalence of 25(OH)D deficiency in healthy children and in critically ill children.

Vitamin D deficiency has recently been shown to be associated with mortality in critically adults. Other recent investigation have not observed this realtionship. Rey et al reported a high prevalence of 25(OH)D deficeincy in critically ill children but not asscociated with higher prediction of mortality risk scores, length of stay and inotropic or respiratory support. Ripple et al reported no asscociation were observed between vitamin D status and predicted PRISM III and PIM 2 mortality. However, Madden et al and Mc Nally et al demonstrated that 25(OH)D level at admission were inversely asscociated with PRISM III in North American children. Ventilator days or non-invasive ventilation and length of PICU stay did not show differences between low and normal 25(OH)D groups in the present sample, in agreement with the data observed by Rippel et al. McNally reported an association of vitamin D deficiency with longer length of stay. Geographic and ethnics differences, as well as the different causes of PICU admissions could explain the similar results in the present study and in the Australian study, and the differences with both North American studies. (12)

The limitations of the study was we did not measure parathyroid hormone (PTH) level. The diagnosis of vitamin D deficiency usually requires the ascociation of serum 25(OH)D levels lower than 20 ng/mL and elevated serum PTH concentrations. (25) We had relative small sample size and the low mortality limited the capacity to analyze spesific subgroup of patients.

In conclusion, in a population of children from Yogyakarta, Indonesia, there was high prevalance of 25(OH)D deficiency. It's not asscociated with PELOD score, mortality rate and PICU length of stay. Further studies are required to identify markers of vitamin D status and its correlation with patient outcome in the intensive care setting.

Table 1. Characteristics of patients based on stratification of 25(OH)D levels on admission

Variable	25(OH)D deficiency n=23	25(OH)D insufficiency n=9	25(OH)D normal n=10	p
Age (months), median (min-max)	27 (1-168)	36 (5-132)	13 (4-74)	0.40
Gender (male), n (%)	15 (65.20)	4 (44.40)	3 (30.00)	0.15
Nutritional state				0.11
- Good, n (%)	14 (60.90)	3 (33.30)	8 (80.00)	
- Malnourished, n (%)	9 (39.10)	6 (66.70)	2 (20.00)	
Ventilator support >48 hours, n (%)	17 (73.90)	9 (100.00)	10 (100.00)	0.06
Procalcitonin level, median (min-max)	15.89 (0.08-200.00)	4.64 (0.08-200.00)	17.17 (0.17-75.60)	0.50

Legend: 25(OH)D=25-hydroxyvitamin D

Table 2. Comparisons of 25(OH)D level among different admission state of sepsis groups and different underlying infections

Variable	25(OH)D deficiency n=23	25(OH)D insufficiency n=9	25(OH)D normal n=10	p
Admission state				0.55
- Sepsis, n (%)	6 (26.10)	3 (33.33)	2 (25.00)	
- Severe sepsis, n (%)	5 (21.70)	3 (33.33)	4 (50.00)	
- Septic shock, n (%)	12 (52.20)	3 (33.33)	2 (25.00)	
Underlying infection				0.21
- Neurology, n (%)	7 (30.40)	3 (33.33)	3 (30.00)	
- Respiratory, n (%)	3 (13.00)	4 (44.44)	5 (50.00)	
- GIT, n (%)	5 (21.70)	0 (0.00)	1 (10.00)	
- Misc, n (%)	8 (34.80)	2 (22.22)	1 (10.00)	

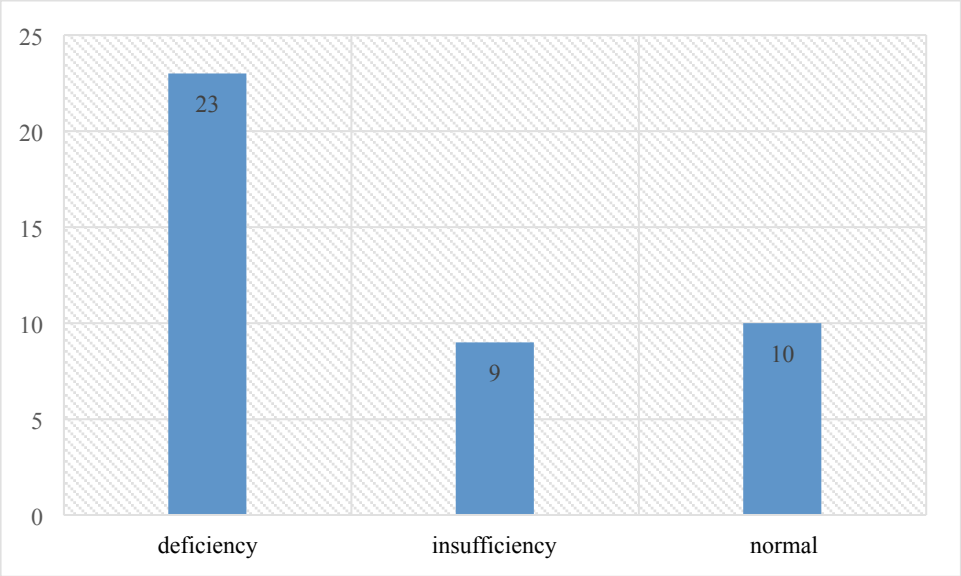
Legend: 25(OH)D=25-hydroxyvitamin D; GIT=gastrointestinal tract

Table 3. Comparison of outcome based on level of 25(OH)D

Outcome	25(OH)D deficiency n=23	25(OH)D insufficiency n=9	25(OH)D normal n=10	p
PELOD score, median (min-max)	20.00 (7.00-55.00)	13.00 (9.00-37.00)	21.50 (6.00-39.00)	0.50
LOS PICU, median (min-max)	10.00 (1.00-30.00)	6.00 (2.00-15.00)	10.00 (5.00-35.00)	0.16
Mortality, n (%)	7 (30.44)	2 (22.22)	2 (20.00)	0.78

Legend: 25(OH)D=25-hydroxyvitamin D; PELOD=pediatric logistic organ dysfunction; LOS=length of stay; PICU=pediatric intensive care unit

Figure 1. Number of patients based on 25(OH)D levels (in ng/ml) on admission



Legend: 25(OH)D=25-hydroxyvitamin D

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