

## Comparison of 25-Hydroxyvitamin D Levels in Pediatric Hematologic Cancer with and without Suspected Sepsis

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### ABSTRACT

Vitamin D is known to play an important role in fighting infections through innate and adaptive immune systems. Children with cancer have a higher risk of suffering sepsis and vitamin D deficiency. Some studies on vitamin D levels in pediatric cancer with sepsis have shown varied results and remain controversial. This study aimed to analyze 25-hydroxyvitamin D levels in pediatric hematologic cancer with and without suspected sepsis. The study was an observational analytical study with a cross-sectional design. Samples were collected during April–September 2019 from the Pediatrics Outpatient Clinic of the Dr. Soetomo Hospital, Surabaya. The samples were a group of pediatric hematologic cancer consisted of 32 subjects with suspected sepsis and 30 without sepsis. Each group was measured the 25-hydroxyvitamin D levels, then differences in levels of 25-hydroxyvitamin D between groups were assessed. Vitamin D levels were measured using ADVIA Centaur. Both groups had low 25-hydroxyvitamin D levels with a deficiency state of 96.9% and 80% in the suspected sepsis group and group without sepsis. There were differences in levels of 25-hydroxyvitamin D in pediatric cancer with and without suspected sepsis ( $p=0.045$ ). Lower vitamin D levels were found in the cancer group with suspected sepsis. Low vitamin D levels reduce T-helper 2 activity, decrease cellular immunity, and decrease phagocytic macrophages, leading to an easier entry of pathogens and bacteremia. There were significant differences in 25-hydroxyvitamin D levels in pediatric hematologic cancer with and without suspected sepsis.

**Keywords:** 25-hydroxyvitamin D, pediatric cancer, sepsis

### INTRODUCTION

Children with cancer have a high risk of sepsis because they are immunocompromised.<sup>1</sup> According to Union for International Cancer Control (IICC), there were 176.000 children diagnosed with cancer every year, and Riskesdas 2018 showed that the prevalence of cancer in Indonesia reaches 4.9% per 1,000 population, and cancer is ranked seventh cause of death.<sup>2,3</sup> Widiaskara's study noted that acute leukemia in children was ranked as the major cause of malignancies in children from 1991-2000 in Dr. Soetomo Hospital, Surabaya, totaling 524 cases or 59% of all malignancies.<sup>4</sup> The average sepsis incidence was 1465 per 100.000 cancer patients compared to 150 per 100,000 patients without cancer.<sup>5</sup>

The acute complication in cancer patients is neutropenic sepsis. Delay in the administration of broad-spectrum antibiotics for one or two hours can worsen the situation; therefore, a fast and accurate diagnostic test is needed to determine sepsis. Biomarkers that have been extensively investigated

for diagnosing sepsis include CRP, IL-2, IL-6, TNF, and procalcitonin, although no specific and sensitive biomarker has been found for the diagnosis of sepsis. Culture remains the gold standard for the diagnosis of sepsis; however, its results take 2-5 days and there are many false-negative results.<sup>6</sup>

Recently, many studies have examined the levels of 25-hydroxyvitamin D in cancer patients and sepsis. Several studies have revealed that children with cancer have low levels of 25-hydroxyvitamin D due to lack of sun exposure, lack of food intake containing vitamin D, and the influence of drugs consumed.<sup>7</sup> However, some studies of vitamin D in pediatric patients with sepsis demonstrated both increased and decreased vitamin D levels. Until now, the role of 25-hydroxyvitamin D as a marker of sepsis in childhood cancer with sepsis is unclear.

Vitamin D has a crucial role in many physiological functions. The main extracellular role of vitamin D is to maintain the balance of calcium, phosphate, and bone mineralization. Recently there has been a lot of research focusing on new effects of vitamin D, such as glucose metabolism, endothelial function, and

immune modulation associated with sepsis. The mechanism of vitamin D in enhancing immunity is complex, this mechanism may have a significant role in the optimum function of the innate immune system by stimulating antimicrobial peptides in epithelial cells, neutrophils, and macrophages. 1,25-dihydroxy vitamin D<sub>3</sub>, which is produced in kidneys, is also generated by immune system cells, mainly during infection.<sup>8</sup> Culic revealed a deficiency of vitamin D status in pediatric cancer, Ahmed in Rajashtan and Tjowanta in Medan revealed a low level of 25-hydroxyvitamin D in pediatric sepsis patient, but the study of Aydemir in Turki revealed a high level of 25-hydroxyvitamin D in pediatric sepsis patient.<sup>9-12</sup> To clarify this situation, this study aimed to analyze 25-hydroxyvitamin D levels in pediatric hematologic cancer with and without suspected sepsis.

According to WHO, cancer in children can strike all ages, races, and genders without known exact causes, only a few are known to be caused by environmental factors or lifestyle factors. Some data, however, states that 10% of children with cancer are caused due to genetic factors.<sup>13</sup>

## METHODS

This study was an observational analytic study with a cross-sectional study design. The samples were collected in April–September 2019 according to inclusion criteria and sample size. The subjects consisted of children diagnosed with hematologic cancer and suspected sepsis and children diagnosed with cancer but without sepsis in the Pediatric Hematology-Oncology Outpatient and Wards, Department of Pediatrics, Dr. Soetomo Hospital, Surabaya. Suspected sepsis patients were identified based on SIRS criteria and at least one positive result of CRP or procalcitonin. The patient without any sign of SIRS criteria was categorized as a non-sepsis group. Children were diagnosed with hematologic cancer (acute lymphoblastic leukemia, acute myeloid leukemia, chronic myelogenous leukemia, and lymphoma) based on bone marrow aspiration according to WHO criteria.

25-hydroxyvitamin D levels were determined using antibody competitive chemiluminescence immunoassay (ADVIA Centaur, Siemens, PT. Tawada Healthcare) in the Clinical Pathology Laboratory, Dr. Soetomo Hospital, Surabaya.

Statistical analysis was demonstrated using SPSS ver 20.0. Mann-Whitney test was used to analyze the difference of proportion between categorical variables. A p-value < 0.05 was classified as statistically significant.

This study was approved by the Research Ethics Committee of Dr. Soetomo Hospital, Surabaya with ethical clearance number 1192/KEPK/V/2019.

## RESULTS AND DISCUSSION

The results of this study that the sample consisted of 32 subjects with cancer and suspected sepsis and 30 subjects with cancer but without sepsis. The median age in suspected sepsis was 6 years old and without sepsis was 5 years old with a higher number of male compared to female subjects. The type of cancers was ALL, AML, CML and lymphoma (Table 1).

All patients with suspected sepsis had performed bacterial culture and CRP or procalcitonin. Only 9 of this patient group were positive in bacterial culture. The median of CRP was 12.8 mg/dL and the median of procalcitonin was 2.7 ng/mL (Table 2). The diagnosis of suspected sepsis in this study were patients who met SIRS criteria, namely 2 of 4 criteria (temperature < 36°C or > 38°C, tachycardia or bradycardia, tachypnea leukocyte > 10.000/uL or < 3000/uL or 10% immature granulocyte) and accompanied by biological signs of infection such as CRP > 10 mg/L or procalcitonin > 0.3 ng/mL or positive culture results.

The median level of 25-hydroxyvitamin D in the suspected sepsis group was 11.185 ng/mL and without sepsis was 13.735 ng/mL. There were significant differences in 25-hydroxyvitamin D levels between the suspected sepsis group and the control group with p=0.045 (Mann-Whitney test p < 0.05) (Table 3). All of the patient group, suspected sepsis and without sepsis group had a low level of 25-hydroxyvitamin D, but the patient group with suspected sepsis had the lowest levels of 25-hydroxyvitamin D. Haan *et al.* revealed that vitamin D deficiency was associated with increased infections, sepsis, 30-day mortality in critically ill patients throughout the world.<sup>14</sup> Another study by Moromizato *et al.* in Boston for 13 years with 3,386 samples of sepsis patients showed that the threshold value of 25-hydroxyvitamin D was smaller or equal to 16 ng/mL associated with the incidence of sepsis and vitamin D deficiency before hospitalization was a significant predictor of sepsis in critically ill people.<sup>15</sup>

Vitamin D is an important role in innate immune stimulation by inducing the synthesis of antimicrobial peptides in innate immune cells, relieving excessive inflammation, and inhibiting the production of proinflammatory cytokines.<sup>16</sup> Many studies report that 1,25(OH)<sub>2</sub>D<sub>3</sub> improves the antimicrobial properties of monocytes and macrophages, also increases the chemotaxis and phagocytic ability of macrophages. 1,25(OH)<sub>2</sub>D<sub>3</sub> also activates the cathelicidin (CAMP) gene

**Table 1.** The characteristics of subjects

	Hematologic Cancer	
	Suspected Sepsis	Without Sepsis
<b>N</b>	32	30
<b>Gender</b>		
Male	21 (65.6%)	19 (63.3%)
Female	11 (34.4%)	11 (36.7%)
<b>Age</b>		
Median	6	5
Mean±SD	7.83±5.43	6.48±4.17
Minimum	0.25	2
Maximum	16	16
<b>Type of cancer</b>		
ALL	17	28
AML	9	1
CML	1	-
Lymphoma	5	1

and-defensin's expression, which are both antibacterial peptides that can destroy microbial cell membranes to prevent infection.<sup>17</sup>

Contrastingly, a study by Aydermir in Turkey in ICU pediatric patients showed a very high level of 25-hydroxyvitamin D levels in pre-treatment patients and decreased post-treatment. Aydemir hypothesized that 25-hydroxyvitamin D increased the production of antimicrobial peptides and contributed to the body's mechanism system, but patients with 25-hydroxyvitamin D deficiency were unable to increase 1,25-dihydroxyvitamin D and indirectly-defensins and cathelicidin, enabling the process of the body's mechanism infection to cause higher morbidity and mortality.<sup>8</sup>

The other results from this study, researchers grouped 25-hydroxyvitamin D levels into vitamin D sufficiency status (> 30 ng/mL), vitamin D insufficiency (20-29 ng/mL), and vitamin D deficiency

**Table 2.** Characteristic of culture, CRP, and procalcitonin

	Hematologic Cancer
<b>Positive culture</b>	9
Gram +	4
Gram -	7
<b>CRP (mg/dL) (normal 0-1 mg/dL)</b>	
Median	12.8
Minimum	1.70
Maximum	31.50
<b>Procalcitonin (ng/mL) (normal &lt; 0.05 ng/mL)</b>	
Median	2.7
Minimum	0.65
Maximum	65

**Table 3.** Mann-Whitney test for different levels of 25-hydroxyvitamin D

	Group	n	Median (min-max)	p-value
Hematologic cancer	Sepsis	32	11,185 (4.2-20.05)	0.045
	Without sepsis	30	13,735 (4.2-34.89)	

**Table 4.** Mann-Whitney test for different status of vitamin D

	25 OH-Vitamin D	Sepsis	without Sepsis	Total	p-value
Hematologic cancer	< 20	31 (96.9%)	24 (80%)	55 (88.7%)	0.035
	20-29	1 (3.1%)	4 (13.3%)	5 (8.1%)	
	≥ 30	0 (0%)	2 (6.7%)	2 (3.2%)	

(<20 ng/mL). There were significant differences in vitamin D status between suspected sepsis and without sepsis groups with  $p=0.035$ . (Table 4).

The most widely used criteria for determination of vitamin D status is those according to the Endocrine Society Advocates, which is < 20 ng/mL for the general population, and there has not been a consensus for the cut-off value of vitamin D deficiency in infants and children until now.<sup>18,7</sup> The experts recommend higher 25-hydroxyvitamin D levels for the immune disease.<sup>19</sup>

Many studies in various countries show that 70-97% of healthy individuals are identified as vitamin D deficiency.<sup>20</sup> The study of the prevalence of vitamin D deficiency in Indonesia was first conducted by the SEANUTS study in 2013 with a sample of 276 healthy children aged 0.5-12 years in various regions in Indonesia. The prevalence of vitamin D deficiency was 44% with slightly higher occurrence in females, probably due to less exposure to sunlight.<sup>21</sup> According to the SEANUTS study, nearly 50% of children in Indonesia suffer from vitamin D deficiency even though Indonesia is rich in sunlight. Also, this study used a population of research samples of children who had cancer. Children with cancer certainly have many risks that contribute to vitamin D deficiency such as reduced intake due to lack of appetite, weakness, and pain that cause more indoors and possibly low levels of active vitamin D associated with kidney disorders and liver due to illness or because of chemotherapy drugs.<sup>22</sup>

Cancer itself has an impact on vitamin D metabolism. Vitamin D receptor (VDR) expression can undergo changes in various cancers and correlates with survival, VDR gene expression can be regulated into genetic changes, which can cause reduced transcription activity depending on 1,25 hydroxyvitamin D Polymorphisms of certain VDR genes have been reported as factors that contribute to genetic susceptibility to vitamin D deficiency, which may exacerbate pre-existing insufficiency in cancer individuals.<sup>23</sup>

The limitation of this study was no consideration of intake of foods containing vitamin D3 and D2, drugs, and the duration of exposure to sunlight, which might cause bias in this study and unknown baseline vitamin D data in the study subjects.

## CONCLUSION AND SUGGESTION

All of the subject's patient hematologic cancer had a low level of 25-hydroxyvitamin D, but patients with suspected sepsis had lower levels of 25-hydroxyvitamin D. There were differences in 25-hydroxyvitamin D levels in pediatric patients

suffering from hematologic cancer with and without suspected sepsis. Further studies were needed to consider food intake and sun exposure, which can cause research bias, and measure 25-hydroxyvitamin D levels in cancer patients to determine baseline values.

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