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# Bali Medical Journal

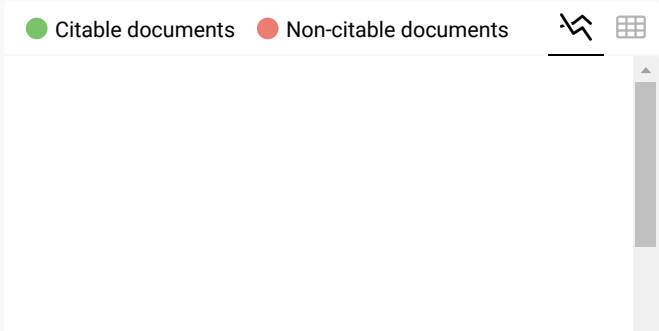
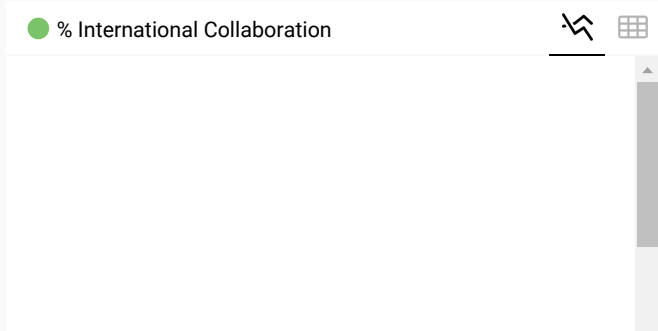
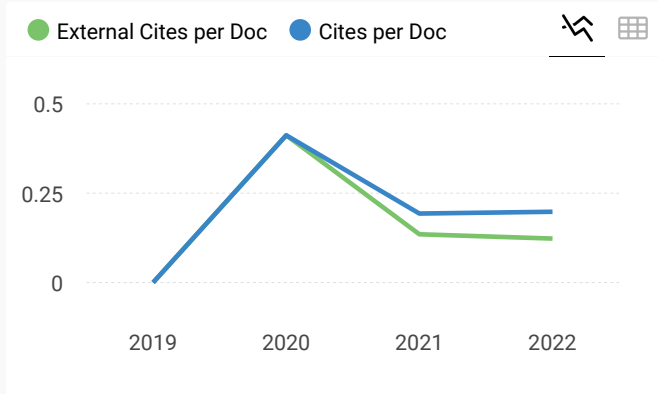
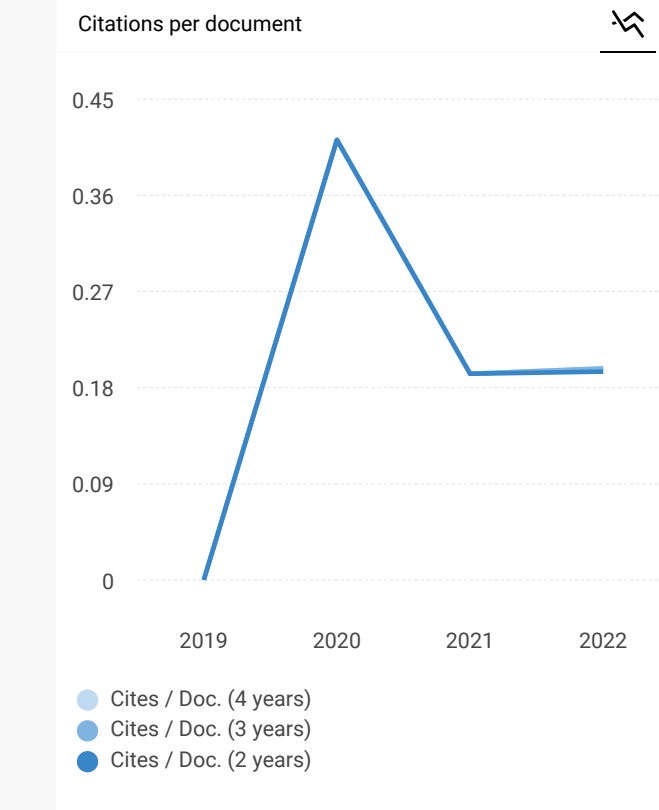
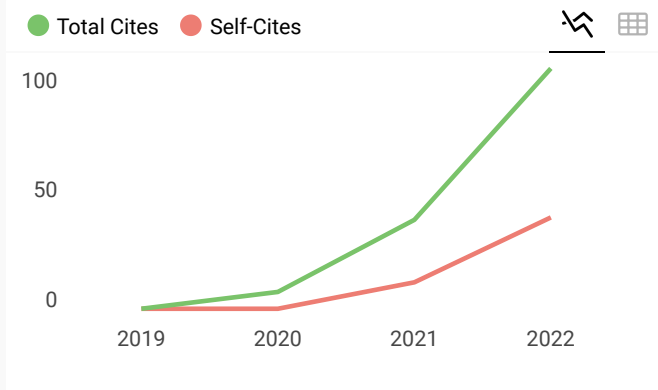
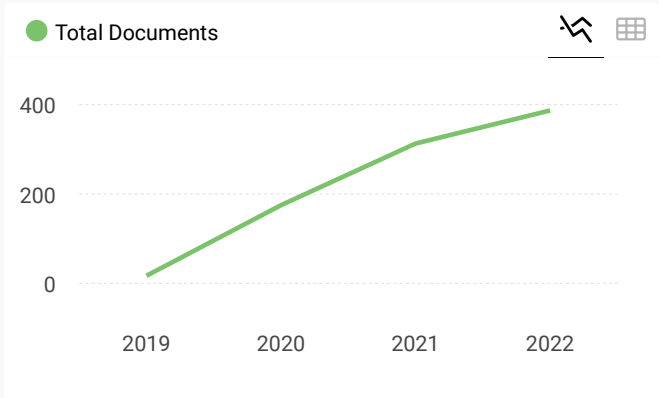
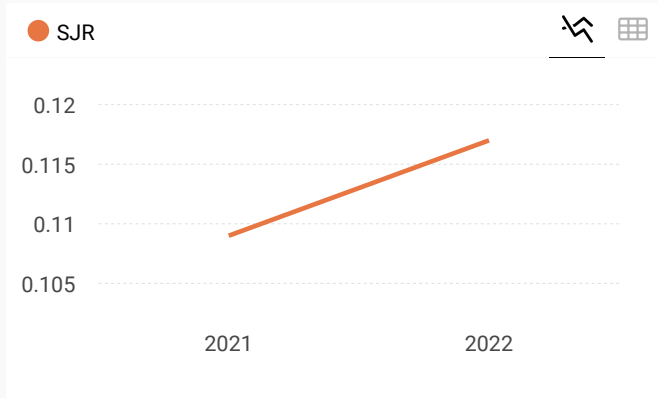
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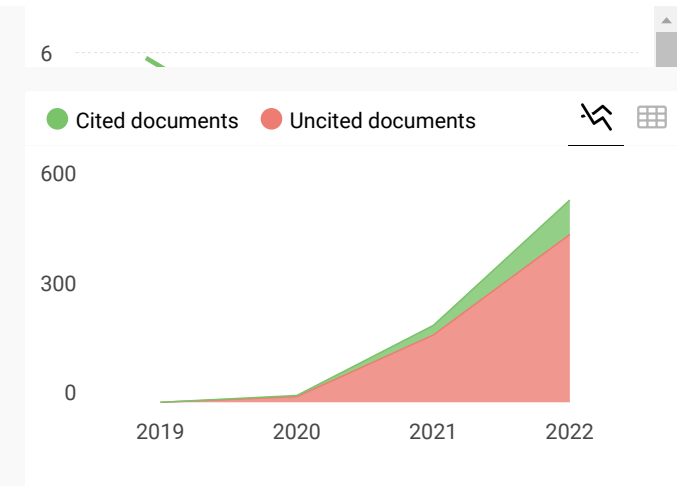
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## Vitamin D and cathelicidin assessment in children with pneumonia



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Fatimah Arief<sup>1,3\*</sup>, Retno Asih Setyoningrum<sup>1,3</sup>, Roedi Irawan<sup>1,3</sup>, Dominicus Husada<sup>1,3</sup>,  
I Ketut Alit Utamayasa<sup>1,3</sup>, Hartono Kahar<sup>2,3</sup>

### ABSTRACT

**Introduction:** Pneumonia is a lung infection that becoming one of the most causes of death in children in developing countries. A deficiency of vitamin D has been suggested in several research had a potential role in reducing cathelicidin production. It is a peptide that could act as an anti-microbial agent. Thus this research aimed to analyze the effect of vitamin D supplementation on serum cathelicidin levels.

**Method:** This study was a quasi-experimental, pretest-posttest control group double-blinded design conducted in the pediatric inpatient ward of General Hospital Dr. Soetomo Surabaya. The inclusion criteria of the sample were children that have been diagnosed with pneumonia clinically, and the ages must range from 1 month up to 5 years old. An oral single dose of 100,000 IU of vitamin D (cholecalciferol) has been administered to the participant, on the day of enrolment after the collection of the blood samples. Cathelicidin and calcidiol (25-hydroxyvitamin D) levels were obtained. Supplementation with oral vitamin D3 (cholecalciferol) at 100,000 IU single dose and placebo was given. At 8 days, all subjects had repeat serum cathelicidin and vitamin D levels. All of the data were analysed in SPSS ver 25. We analyze the data descriptively and analytically. For the analytic analysis, we analyze the data by man-witney and paired t-test.

**Results:** Forty-six subjects were consisting in this study consisting of 23 cases and 23 controls, 30/46 were males and 16/46 were females. The median age in the study was 24 months. The mean levels of cathelicidin in the post-supplementation treatment group were 11.80 + 11.40 with p value= 1.00. Meanwhile, the mean level of cathelicidin in the placebo group after giving the placebo was 8.81 + 6.46 with p value= 0.29.

**Conclusion:** Enhancement of vitamin D in children with pneumonia after giving the supplementation has been established. Meanwhile, only calcidiol serum level was to be significant in difference when comparing the pre and post-examination of the vitamin D supplementation group.

**Keywords:** cathelicidin, children, pneumonia, vitamin D.

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### INTRODUCTION

Inflammation of lung parenchyma by bacteria is known as pneumonia. It is becoming the most common cause of mortality and morbidity among children, particularly in developing countries.<sup>1</sup> a 15% mortality rate among children in Indonesia due to pneumonia.<sup>2</sup> The high prevalence of pneumonia in east Java has been reported at 78.81% in 2010.<sup>3</sup> Other research has established, that 15% of all deaths in children below 5 years of age.<sup>4</sup>

Treatment for pneumonia includes antibiotics, supplementary oxygen, and other supportive therapies. There was a relationship between vitamin D deficiency with infection in the respiratory tract. Vitamin D has a pivotal role in boosting

immune defenses and reducing excessive inflammation. That mechanism could help the children to recover from an acute episode of pneumonia.<sup>5</sup> A 1.25(OH)2D was able to induce cathelicidin. Cathelicidin act as an antimicrobial peptide that is expressed in human respiratory epithelial cells.<sup>6</sup>

Thus in this study, we aimed to give an oral single dose (100,000 IU) of vitamin D supplementation for pneumonia along with the estimation of baseline and post-intervention serum 25(OH)D levels, and human peptide cathelicidin.

### METHOD

#### Study design

This study was a quasi-experimental, pretest-posttest control group double-

blinded design conducted in the pediatric inpatient ward of General Hospital Dr. Soetomo Surabaya. Informed consent was taken from the caregivers.

#### Participants

The inclusion criteria for this study were aged 1 month- 5 years, diagnosed with pneumonia by a respiratory pediatric consultant and obtained permission from their family to participate in the study. Exclusion criteria included: having taken vitamin D supplements 4 weeks before the study, children having received antibiotics more than 3 days before the study started, children with cyanotic congenital heart disease, kidney failure, and acute post-streptococcal glomerulonephritis, a history of allergy to vitamin D.

### Randomization and masking

Eligible children were randomly assigned to receive either 100,000 IU of vitamin D3 or placebo orally using computer-generated block randomization. In terms of appearance, color, odor, amount, and taste, the drug and placebo were identical. Pharmacy staff worked independently on randomization, repackaging, sequencing, and allocation concealment. In this study, 46 participants were recruited, with 23 from the intervention group and 23 from the control group.

### Baseline data collection

The vital signs examination has been done by the health practitioner such as temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation. Other sign that has been noticed such as cyanosis, nasal flaring, grunting, and mental status. Weight, length/height, and mid-upper arm circumference were recorded for all participants as per standard techniques. Weight-for-age Z-score (WAZ), height/length-for-age Z-score (HAZ), and weight-for-height/length Z-score (WHZ) were derived using the WHO growth chart.

### Intervention

On the day of enrollment, a single dose of 100,000 IU of vitamin D (cholecalciferol) was administered orally or via nasogastric tube to the participant after blood was taken. Participants were treated by a standard procedure. Measures were taken upon admission to establish and maintain a patent airway, breathing, and circulation.

### Investigations

Cathelicidin and calcidiol (25-hydroxyvitamin D) levels were obtained. Supplementation with oral vitamin D3 (cholecalciferol) at 100,000 IU single dose and placebo was given. At 8 days, all subjects had repeat serum cathelicidin and vitamin D levels.

### Statistic analysis

All of the data were analyzed in SPSS ver 25. We analyze the data descriptively and analytically. For the analytic analysis, we analyze the data by using man-witney and paired t-tests

## RESULTS

### Baseline Characteristics

According to our research, we found the median age of the placebo group was 24 months old, and the vitamin D supplemental group was 9 months old (Figure 1). According to gender, the proportion was similar either in the placebo or in the vitamin D supplemental group. In the last intervention, we assessed the calcidiol and cathelicidin levels. In this assessment, we found the higher level of cathelicidin level in the vitamin D supplemental group (11.63 ng/dL) rather than in the placebo group ( 8.18 ng/dL). In contrast, calcidiol level was higher in the placebo groups (28.16 ng/dL), rather than in the vitamin D supplemental group (27.27 ng/dL). According to the analytic assessment, we found that there was no significant difference between the placebo group and the vitamin D supplement group.

According to the analytic analysis, we found a significant relationship between giving vitamin D supplementation in the enhancement of calcidiol levels pre and post-intervention of the vitamin d supplementation group ( $p=0.04$ ). Meanwhile, the placebo group also showed

the enhancement of calcidiol levels from the pre and post-intervention, but no sign of difference (table 2).

In contrast with calcidiol outcome (table 2), for the cathelicidin analytic analysis, we did not find any significant difference between pre in post-intervention in each group ( $p>0.05$ ). The enhancement of cathelicidin levels in both groups was seen descriptively, meanwhile, it was only a view enhancement.

## DISCUSSION

The immune system and clinical response to vitamin D may vary for bacterial or viral illnesses.<sup>7</sup> This theory also has been proven by a meta-analysis. Several studies involved in the meta-analysis established different mean vitamin D (Mean 25(OH) D/mL) levels in serum. According to six studies, only one study showed a higher vitamin D level in a patient with community-acquired pneumonia (CAP) rather in non-CAP. Implicitly these results explain that the differences in infecting germs also affect vitamin D, and also have an impact on the response of the immune system. Of course, this will also have an impact on patient outcomes.<sup>8</sup>

In this study, the mean serum levels of

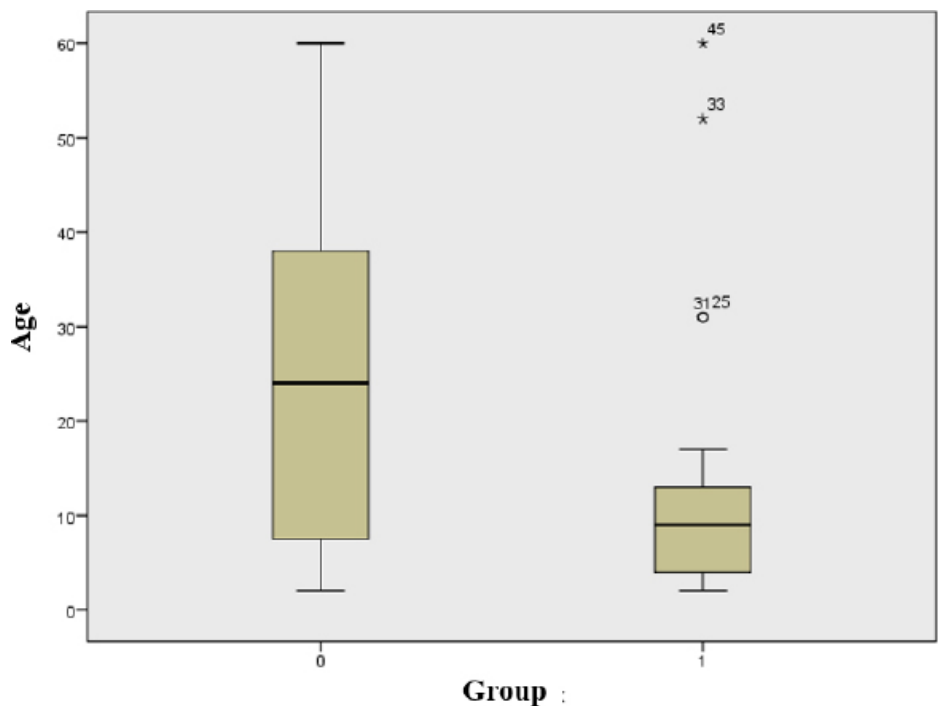


Figure 1. Age profile in the two study groups.

vitamin D in the vitamin D supplemental group were higher than the control group but the levels of cathelicidin between the 2 groups were not significantly different. Buonfiglio study i.e. found an enhancement of cathelicidin levels through vitamin D supplementation (Buonfiglio, 2017).<sup>7</sup> Other research related to pneumonia and vitamin D in Indonesia has been done among 2-59 months old with pneumonia. The initial level of vitamin D level was varied. Several cut off of vitamin D level has been determined in several conditions. From 133 children it established that the cut-off vitamin D level < 75 nm/L<sup>2</sup>, <50 nm/L<sup>2</sup>, or <25 nm/L<sup>2</sup> did not have any relationship with the danger sign of pneumonia, hypoxemia, and

prolonged stay of patients with pneumonia (p>0.05). The researchers conclude that even though vitamin D level in children with pneumonia does not always have a low level of vitamin D serum. It also cannot be used as a determinant of the outcome of the patient. Thus, vitamin D supplements are not necessarily a crucial thing to give to children with pneumonia.<sup>9</sup> In addition, similar research with 90 children aged 6 months old – 3 years old established no significant difference in vitamin D levels in the control group compared with the pneumonia group. Interestingly, vitamin D level in the pneumonia group was higher rather in the control group. Meanwhile, it was not significant in difference. Thus, Vitamin D supplementation needs to be

re-examined.<sup>10</sup>

Vitamin D signaling is required for innate defense against intracellular microorganisms by generating the antimicrobial protein cathelicidin.<sup>11,12</sup> The mechanism of vitamin D in pneumonia was extensively known. Binding to the vitamin D receptor (VDR) can activate 1, 25-(OH)2D3. In addition binding to its receptor also can stimulate the production of antibacterial peptides. Thus it can help against the pathogen. In contrast, when the level of vitamin D is low it could reduce the VDR, and certain mechanisms against the pathogen would not happen. Unfortunately, vitamin D deficiency is often found in pneumonia among children. Another, possible mechanism act by the vitamin D could neutralize the inflammation, thus it could prevent the worsening condition. Thus, administering vitamin D supplementation might reduce the occurrence and the severity of CAP.<sup>8</sup>

In addition to directly binding to and killing a range of pathogens, cathelicidin acts as a secondary messenger driving vitamin D-mediated inflammation during infection.<sup>11,12</sup> Subanada study to establish whether vitamin D deficiency, ff genotype-Fok1 VDR polymorphism, and cathelicidin level are risk factors of acute lower respiratory infections ALRIs and to determine the pictures of exon 2-VDR genes polymorphisms in children under five. It is indicated that vitamin D deficiency and low cathelicidin levels are risk factors, but ff genotype-Fok1 VDR gene polymorphism is not (ALRIs).<sup>13</sup>

**Table 1. Baseline characteristics of study participants.**

Variable	Placebo Group n = 23	Vitamin D Supplemental Group n = 23	p*
Age (mo), median (min-max)	24 (2-60)	9 (2-60)	0.074
Age range (mo), n (%)			
1-12	9 (39.1)	17 (73.9)	
13-60	14 (60.9)	6 (26.1)	
Gender, n (%)			
Male	15 (65.2)	15 (65.2)	1.00
Female	8 (34.8)	8 (34.8)	
Nutritional Status, n (%)			
Severe Malnutrition	6 (26)	6 (26)	0.667
Moderate Malnutrition	4 (17.4)	2 (8.6)	
Normal	13 (56.6)	15 (65.4)	
Overweight/Obesity	0 (0)	0 (0)	
Calcidiol, ng/dL (mean)	28.16	27.27	0.70
Cathelicidin, ng/dL (mean)	8.18	11.63	0.49

\*man-witney test

**Table 2. The outcome of calcidiol (25 (OH) vitamin D) in the two study groups.**

Group	Placebo Group n = 23				Vitamin D Supplemental Group n = 23			
	Pre	Post	CI 95%	p*	Pre	Post	CI 95%	p*
	Mean ± SD	Mean ± SD			Mean ± SD	Mean ± SD		
Calcidiol Level (ng/dL)	28.16 ± 9.03	30.70 ± 19.00	-5.92 – 10.99	0.90	27.27 ± 9.14	53.08 ± 64.5	-1.47 – 53.09	0.04

\*paired t-test analysis

**Table 3. The outcome of cathelicidin in the two study groups.**

Group	Placebo Group n = 23				Vitamin D Supplemental Group n = 23			
	Pre	Post	CI 95%	p*	Pre	Post	CI 95%	p*
	Mean ± SD	Mean ± SD			Mean ± SD	Mean ± SD		
Cathelicidin Level (ng/dL)	8.18 ± 9.03	8.81 ± 6.46	-4.21 – 5.47	0.29	11.63 ± 12.29	11.80 ± 11.40	-4.21 – 5.47	1.00

\*paired t-test analysis

The limitation of our study are we did not assess the microbiological diagnosis of pneumonia by lung tap or bronchoalveolar lavage, thus we were unable to assess the response of vitamin d in each of the different types of bacteria. The severity of pneumonia did not assess in this study, it might have an impact on the outcome of giving vitamin d supplementation. Cell-mediated immunity is known to have an impact on vitamin D supplementation administration, but there are limited funds.

## CONCLUSION

Enhancement of vitamin D in children with pneumonia after giving the supplementation has been established. Meanwhile, only calcidiol serum level was seen to be significant in difference when comparing the pre and post-examination of the vitamin D supplementation group.

## DISCLOSURE

### Conflict of Interest

None.

### Funding

None.

### Author Contribution

All of the authors contributed to this article.

### Ethical Clearance

The protocol of this study was approved by the Ethics Commission of Dr. Soetomo

General Hospital (0379/KEPK/III/2022). The study was conducted at the Pediatric Ward, Department of Child Health, Dr. Soetomo General Hospital Surabaya from March to May 2022.

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