

Original Article

Serum 25-Hydroxy Vitamin D Levels in Japanese Infants with Respiratory Syncytial Virus Infection Younger than 3 Months of Age

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SUMMARY: Low blood levels of vitamin D have been reported in children who have frequent respiratory tract infections. We measured serum concentrations of 25-hydroxy (OH) vitamin D in Japanese infants under 3 months of age who had respiratory syncytial virus (RSV) infection. Serum levels of 25-OH vitamin D in the 10 infants, excluding those with underlying diseases, were between < 4 and 29.8 ng/mL. In 8 out of 10 subjects (80.0%), serum 25-OH vitamin D levels were lower than 20 ng/mL. There was no statistically significant association between the levels of 25-OH vitamin D and age, duration of admission, respiratory severity score, white blood cell count, blood gas levels, and N-terminal pro-natriuretic peptide levels. Levels of serum 25-OH vitamin D in children who required hospitalization owing to RSV infection were low, indicating deficiency. These results suggest that vitamin D deficiency affects the susceptibility to RSV infection, but not the severity of the infection.

INTRODUCTION

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections in Japan. It results in clinical diseases, such as bronchiolitis and pneumonia, and is the most common cause of hospitalization among infants (1). Vitamin D deficiency has been reported to correlate with RSV infection, as the blood concentration of 25-hydroxy (OH) vitamin D is associated with the incidence of RSV infection in the first year of life, according to studies from foreign countries (2–4). Polymorphisms of the *VDR* gene have also been reported to be associated with a genetic predisposition to RSV bronchiolitis (5,6). Vitamin D levels in infants differ according to their region of habitation, which affects the duration and amount of sun exposure as well as their eating habits. To clarify the involvement of vitamin D deficiency in RSV infection in young infants in Japan, we measured serum 25-OH vitamin D levels in infants younger than 3 months of age who were hospitalized owing to RSV infection.

MATERIALS AND METHODS

Ten patients (6 boys and 4 girls) aged 0 to 3 months,

who were hospitalized in the Department of Pediatrics, Tokyo Medical University Hospital between January 2017 and January 2019, were enrolled in this study. Patients with congenital heart disease or low birth weight were excluded. All other patients with no serious underlying diseases who were diagnosed with bronchiolitis or pneumonia caused by RSV using a rapid antigen test were included (Check RSV; Alfresa, Osaka, Japan) (Table 1). To evaluate the severity of respiratory symptoms, the respiratory severity score established by Nariai was used (7). Levels of serum 25-OH vitamin D were measured by the chemiluminescent immunoassay (CLIA) method and subjects were categorized into the following 3 groups: Normal (>30 ng/mL), Low (20–30 ng/mL), and Deficient (< 20 ng/mL). Serum 25-OH vitamin D levels of 10 age-matched hospitalized controls (4 with fever of unknown origin, 2 with urinary tract infections, 1 with influenza, 1 with asphyxia, 1 with bronchitis, and 1 with HIV infection) were also measured. N-terminal pro-natriuretic peptide (NT-proBNP) levels were also measured by the CLIA method to investigate possible involvement of the heart in RSV infection, and levels higher than 1,000 pg/mL were defined as extremely high. Renal function data of all subjects were in the normal range, and serum P and Ca levels were also normal in all subjects. Alkaline phosphatase levels of all subjects were lower than 1,000 IU/L. None of the subjects were clinically diagnosed with rickets. Serum samples on admission were subjected for assay. Statistical analyses were performed using IBM® SPSS® Statistics version 25.0 software. A Pearson *r*-coefficient of greater than 0.8 was considered to indicate a statistically significant correlation (*p*-value < 0.05).

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Table 1. Profile of the subjects who were infected RS virus at the age of 3 months or younger

Age (month)	Severity	Disease	Duration of admission	Underlying disease	Asthma after RS infection	Respiratory support	Birth weight (g)	Gestation	25-OH vitamin D (ng/mL)	NT-pro BN (pg/mL)	Respiratory severity score
1	Mild	Bronchiolitis	5		-	None	3,028	40w3d	< 4	325	4
1	Moderate	Bronchiolitis	7		-	→biphasic mode	2,970	40w1d	< 4	562	7
2	Mild	Bronchiolitis	3	Umbilical hernia, quiescent testes, fever at 1 month	-	None	2,978	38w2d	11.5	741	1
2	Mild	Pneumonia	7		-	None	3,184	38w3d	7.2	2,030	3
3	Mild	Bronchiolitis	5		-	None	2,850	39w	16.5	84	6
0	Severe	Bronchiolitis	12		+	Artificial ventilation	2,750	38w6d	17.8	4,090	11
1	Mild	Bronchiolitis	6		-	None	Unknown	Unknown	13	153	5
3	Mild	Bronchiolitis	8		-	O ₂ mask	3,270	39w1d	29.8	137	6
2	Mild	Pneumonia	7	Umbilical hernia	-	O ₂ mask	3,050	38w	25.5	272	3
1	Severe	Pneumonia	27	after TTN, and pneumothorax	-	Artificial ventilation	2,874	36w4d	18.1	229	8

TTN; transient tachypnea of the newborn, CPAP; continuous positive airway pressure.

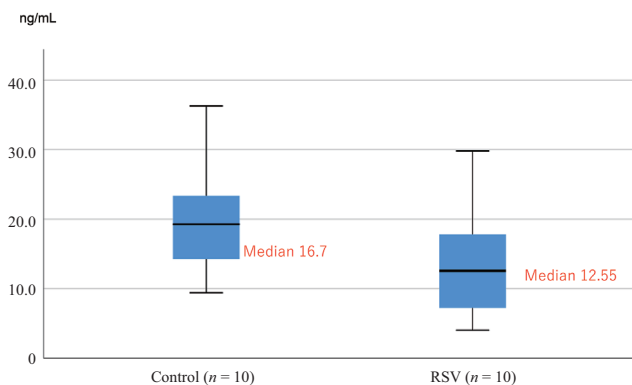


Fig. 1. (Color online) Serum levels of 25-OH vitamin D in patients infected with RSV and controls. Eight out of 10 subjects with RSV infection (80.0%) had serum 25-OH vitamin D levels of less than 20 ng/mL. Six out of the 10 age-matched hospitalized controls (60.0%) showed levels less than 20 ng/mL. The boxes in the graph indicate the first quartile and third quartile, and the bars indicate maximum, the second quartile, and minimum values. Statistical analysis was performed using the unpaired *t*-test. There was no statistically significant difference between the 2 groups (*p* value = 0.538 > 0.05).

This study was approved by the Ethics Review Board of Tokyo Medical University (study approval no.: SH3841). Blood samples were collected from all patients after obtaining informed consent from the parents at the time of the patient's admission.

RESULTS

Serum levels of 25-OH vitamin D among the 10 subjects were between < 4 and 29.8 ng/mL. None of the subjects showed a 25-OH vitamin D level higher than 30 ng/mL, which is considered normal. Eight out of 10 subjects (80.0%) had levels lower than 20 ng/mL, which is defined as a deficiency of vitamin D. The serum levels of 25-OH vitamin D among the 10 subjects

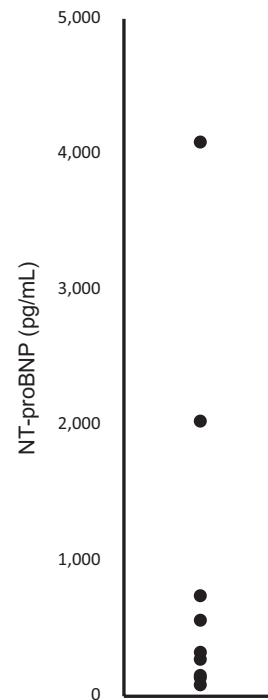


Fig. 2. Serum levels of NT-proBNP in patients infected with RSV. No correlation was found between NT-proBNP and 25-OH vitamin D levels in patients infected with RSV. Two patients showed high levels NT-proBNP.

with RSV infection were lower than those of the age-matched controls. However, the difference between the 2 groups was not statistically significant (Fig. 1). Serum levels of NT-proBNP were significantly higher in 2 of the 10 patients (Fig. 2). The levels of 25-OH vitamin D did not correlate with age (months), the presence of bronchiolitis vs pneumonia, duration of admission, requirement of respiratory support, respiratory severity scores, birth weight, respiratory rates, white blood

cell (WBC) counts, SpO_2 , PCO_2 , HCO_3^- , and NT-proBNP levels. However, respiratory severity scores of RSV infection correlated with duration of admission, PCO_2 , and HCO_3^- levels. NT-proBNP levels correlated with the duration of admission and PCO_2 levels. Levels of 25-OH vitamin D were correlated only with C-reactive protein levels. Data are shown in Table 2.

DISCUSSION

Vitamin D is associated with bone remodeling, and it also plays a role in the improvement in immune function and reduction in inflammation. Recently, vitamin D has been shown to play a key role in the regulation of innate immunity (8). In this study, 8 out of 10 infants who were hospitalized owing to RSV infection showed serum 25-OH vitamin D levels lower than 20 ng/mL, which is classified as a vitamin D deficiency. This deficiency readily occurs due to low levels of vitamin D uptake and a lack of sun exposure, because the circulating half-life of vitamin D is only approximately 15 days (9). Preterm birth, inadequate vitamin D intake, lack of sun exposure, fat malabsorption, obesity, and impaired absorption are considered risk factors for vitamin D deficiency (10). However, in the present study, none of the subjects had these conditions.

Belderbos et al. showed that vitamin D deficiency in healthy neonates was associated with an increased risk of RSV-associated lower respiratory tract infection in the first year of life (3). Halasa et al. investigated the risk factors of severe respiratory symptoms, including low vitamin D levels, in Middle Eastern and Arab countries. RSV-positive children were more likely to be previously healthy and have no other any underlying medical conditions, were less likely to be born prematurely, and had lower median vitamin D levels than the RSV-negative group (2). In a rare study conducted in Japan by Inamo et al., the association between vitamin D deficiency and the severity of respiratory infection was investigated by determining serum 25-OH vitamin D levels in hospitalized children with acute lower respiratory infections, including RSV infection. A significant correlation was found between vitamin D deficiency (<15 ng/mL) and the need for supplementary oxygen and ventilator management (11). However, this study was not focused on RSV infection.

The pathophysiology of vitamin D deficiency has been studied based on both the immunological status and the local defending status. The secretion of the peptides LL-37 and I3-defensin 2, which have anti-viral activity against RSV, is induced by vitamin D, and inhibits the production of new infectious particles and the spread of infection (12,13). Additionally, levels of $1,25\text{-(OH)}_2$ vitamin D, which is the activated form of vitamin D, correlate with the suppression of inflammatory cytokines and the production of IgG. Moreover, in experiments using human airway epithelial cells, it was found that the production of NF- κ B-linked pro-inflammatory cytokines following RSV infection is

Table 2. Pearson correlation coefficients and *p*-values

	Range (mean)	Age (month)	Bronchiolitis vs pneumonia	Duration of admission	Respiratory support	Severity score	Birth weight	RR	WBC	Hg	CRP	SpO_2	PCO_2	HCO_3^-	25-OH vitamin D	NT-pro BNP
Duration of admission (days)	3–27(6.66)	<i>r</i> -0.356 <i>p</i> 0.313	0.5 0.141	1	.771** 0.009	0.565 0.088	-0.328 0.388	0.114 0.755	-0.382 0.276	0.315 0.375	.871** 0.001	-0.451 0.262	.944** 0	.855** 0.002	0.039 0.915	0.084 0.818
Respiratory support	Unnecessary: 1 Required O_2 : 2 Under artificial support: 3	<i>r</i> -0.447 <i>p</i> 0.195	0.251 0.483	.771** 0.009	1 0.011	.760* 0.011	-0.393 0.296	0.516 0.127	-0.205 0.57	0.119 0.743	0.553 0.097	-0.324 0.433	.830** 0.003	.885** 0.001	0.317 0.372	0.379 0.28
Severity score	1–11(5.11)	<i>r</i> -0.496 <i>p</i> 0.145	-0.176 0.627	0.565 0.088	.760* 0.011	1 0.011	-0.556 0.12	0.494 0.147	-0.025 0.945	0.506 0.136	0.366 0.298	-0.521 0.185	.709* 0.022	.708* 0.022	0.115 0.752	0.463 0.178
25-OH vitamin D (ng/mL)	<4–29.8 (14.3)	<i>r</i> 0.459 <i>p</i> 0.182	0.064 0.861	0.039 0.915	0.317 0.372	0.115 0.752	0.219 0.571	0.087 0.811	0.532 0.113	-0.445 0.197	0.216 0.548	-0.399 0.328	-0.004 0.992	0.07 0.849	1 0.883	-0.054 0.883
NT-pro BNP (pg/mL)	137–4,090 (932.6)	<i>r</i> -0.523 <i>p</i> 0.121	-0.01 0.978	0.084 0.818	0.379 0.28	0.463 0.178	-0.36 0.341	0.575 0.082	0.079 0.829	0.028 0.939	-0.272 0.446	0.405 0.32	0.24 0.503	0.14 0.7	-0.054 0.883	1 0.883

P* < 0.05, *P* < 0.01, *r* and *p* represent the Pearson *r*-correlation.

Bold values represent a statistically significant correlation between both markers or factors by SPSS.

inhibited by vitamin D treatment (14).

Although vitamin D is considered to be effective against severe RSV infections, our results did not show any statistically significant correlation between the levels of 25-OH vitamin D and the duration of admission, respiratory severity scores, WBC counts, blood gas levels, and NT-proBNP levels. Beigelman reported that the vitamin D status at the time of bronchiolitis was not associated with indicators of the severity of acute bronchiolitis, consistent with our results. The indicators of acute bronchiolitis severity that did not differ between infants with and without vitamin D deficiency, despite adjusting for age and infant formula consumption, were duration of hospitalization, lowest oxygen saturation, and bronchiolitis severity score (15). In contrast, a study by Hurwitz et al. found that the levels of retinol-binding protein and vitamin D were associated with severe outcomes in hospitalized children with lower respiratory tract infection and RSV infection. Low vitamin D levels were observed in 50% of the children and were associated with a significantly increased risk for the need of admission to an intensive care unit and invasive mechanical ventilation (16).

A systematic review by Eisenhut et al. showed that extra-pulmonary manifestations of RSV infection included cardiovascular failure with hypotension and inotrope requirement associated with myocardial damage, as evident from increased cardiac troponin levels (35–54% of ventilated infants) (17). NT-proBNP is a marker of heart failure resulting from pressure overload in RSV pulmonary infection. NT-proBNP levels in 2 out of the 10 patients were significantly high in the present study, although they showed no correlation with 25-OH vitamin D levels. RSV infection is usually complicated with myocarditis, and patients seldom have serious arrhythmia (18,19). Patients often have mild myocarditis with RSV infection. Further studies to identify potential confounding factors are necessary to conclude the effectiveness of vitamin D in the prevention of RSV infection in infants.

Recent studies have provided information regarding the association between cord blood 25-OH vitamin D levels and the risk of respiratory infections in infants. In a Korean birth cohort study, the authors investigated cord blood vitamin D levels and respiratory tract infection at a 6-month follow-up. The results showed that 34.3% of infants had 25-OH vitamin D concentrations lower than 25.0 nmol/L, and cord blood vitamin D insufficiency or deficiency in healthy neonates was associated with an increased risk of acute nasopharyngitis by 6 months of age (4). Mohamed et al. also reported similar results, that is, an increased risk of acute lower respiratory tract infection in the first 2 years of life (20). Cord blood 25-OH vitamin D concentrations have been found to be strongly associated with maternal vitamin D3 supplementation during pregnancy. Therefore, enhancing routine vitamin D supplementation during pregnancy may be a useful strategy to prevent RSV-associated lower respiratory tract infections during infancy.

A limitation of this study is that the sample size was too small to provide statistically significant results to support our conclusion.

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Conflict of interest None to declare.

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