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Relationship between vitamin D levels and intravenous immunoglobulin resistance in Kawasaki disease

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Purpose: Vitamin D is associated with various pathological conditions such as cardiovascular diseases and cancer. We investigated the relationship between vitamin D and Kawasaki disease (KD).
Methods: We performed a retrospective review of the medical records of patients with KD between February 2013 and March 2016 in Daegu Fatima Hospital. Study participants were grouped according to vitamin D serum concentration. Group 1 included patients with 25(OH)-vitamin D ≥20 ng/mL. Group 2 included patients with 25(OH)-vitamin D <20 ng/mL. We analyzed the clinical characteristics and laboratory data of the 2 groups.
Results: Of the 91 patients, 52 were included in group 1, and 39 in group 2. Group 1 patients had significantly higher levels of calcium, phosphate, albumin and sodium than group 2 patients did. There were no differences in clinical characteristics, but the proportion of patients with polymorphic rash was significantly higher in group 2. Resistance to intravenous immunoglobulin was more frequent in group 2 (P=0.023). No significant difference in the incidence of coronary artery complications was observed.
Conclusion: Low vitamin D levels are associated with resistance to intravenous immunoglobulin therapy in KD. Vitamin D deficiency might be a risk factor for immunoglobulin resistance in KD.

Key words: Vitamin D, Kawasaki disease, Inflammation

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Introduction

Vitamin D is a hormone that maintains calcium homeostasis and participates in bone metabolism. Even though it can be obtained through the diet, it is mostly synthesized in the skin upon exposure to ultraviolet radiation. Vitamin D must be activated in the liver and kidneys to perform its physiological functions. In addition to its role in calcium and bone metabolism[1], vitamin D has been associated with various pathological conditions such as cardiovascular diseases, cancer, and diabetes, as it can act on vitamin D receptors in cells of various human tissues such as the skin, colon, heart, prostate, and blood vessels [2,3]. The concentration of vitamin D in the body is measured based on 25(OH)-vitamin D levels because of its relatively long half-life.

Although studies on vitamin D deficiency have proposed various cutoff values, a serum levels lower than 20 ng/mL is commonly defined as vitamin D deficiency[4-6].

Kawasaki disease (KD) is an acute multisystemic vasculitis primarily affecting infants and young children. Although it entails acute inflammatory injury to all systemic arteries, injuries are particularly severe in the coronary arteries, where dilatations or even aneurysms can be encountered in patients who do not receive adequate treatment. Because the incidence of coronary artery aneurysm is increased by delayed treatment, early diagnosis and treatment is

the most important factor in improving prognosis[7].

Several studies have reported that vitamin D exerts an anti-inflammatory activity. Moreover, low vitamin D levels reportedly increase the incidence of cardiovascular disease and are associated with cardiovascular mortality[8,9].

There are many studies on the effects of vitamin D and its association with various diseases, but rarely on KD. In our study, we investigated the relationship of vitamin D with immunoglobulin resistance, and coronary artery lesions in KD patients.

Materials and methods

1. Patients

The study group comprised patients with KD admitted to Daegu Fatima Hospital, South Korea between February 2013 and March 2016. Cases of incomplete KD were excluded. We investigated 91 patients and retrospectively measured their vitamin D concentrations. KD diagnosis was based on the American Heart Association guidelines[10]. In all patients at the time of admission, the following parameters were measured: leukocyte, and platelet count, hemoglobin levels, hematocrit, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), electrolytes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, total protein, albumin, N-terminal fragment of B-type natriuretic peptide (NT-proBNP), alkaline phosphatase (ALP), and 25(OH)-vitamin D levels. Urine-analyses were also performed, with white blood cell counts ≥5 defined as pyuria.

Echocardiography was performed within 1 week from the onset of fever. We measured the intraluminal diameters of the left main coronary artery (LMCA) and the right coronary artery (RCA). Coronary artery complications were diagnosed per the standard criteria published by the Japanese Ministry of Health and Welfare, according to which the presence of coronary arteries with a diameter of 3 mm or more in children younger than 5 years, 4 mm or more in children aged 5 years or older, or the existence of coronary artery segments with a diameter 1.5 times greater than that of an adjacent segment, constitute coronary artery dilatation[11]. In our study, all coronary measurements were adjusted for body surface area according to de Zozi reference data to avoid misdiagnosis and underestimation of the true prevalence of coronary artery abnormalities[12]. High doses of intravenous immunoglobulin (IVIG; 2 g/kg) and aspirin (80 mg to 100 mg/kg) were administered to all patients. Resistance to IVIG was defined as persistent or recrudescent fever ≥36 hours after completion of the initial IVIG infusion. In the absence of resistance, i.e., when the fever subsided within 36 hours from the completion of the initial IVIG infusion, the dose of aspirin was decreased to 5 mg/kg/day.

2. Study methods

Study participants were grouped according to vitamin D serum concentration. Group 1 included patients with 25(OH)-vitamin D level ≥20 ng/mL, whereas group 2 included those with 25(OH)-vitamin D level <20 ng/mL. We compared the 2 groups with respect to sex ratio, age, clinical characteristics of KD, blood test results, *z* score of LMCA and RCA, the presence of coronary artery complications, and resistance to IVIG therapy.

3. Statistical analysis

All statistical analyses were performed with IBM SPSS ver. 18.0 (IBM Co., Armonk, NY, USA). Student *t* test was used for comparing continuous variables and crossover analysis for the comparison of frequencies and fractions. Results were expressed as mean ±standard deviation and considered statistically significant when $P<0.05$.

Results

Of the 91 patients, 52 were included in group 1 and 39 were included in group 2. The mean age was 30.9±20.7 months in group 1 and 37.1±20.2 months in group 2. Even though the mean age was higher in group 2, but the difference was not statistically significant ($P=0.159$). The proportion of male patients in group 1 (73.1%) was significantly higher (46.2%, $P=0.009$) than that in group 2. Fever duration before KD diagnosis was not significantly different between the two groups (5.7±1.1 days in group 1 and 5.5±1.1 days in group 2, $P=0.461$).

With respect to KD clinical characteristics, there was no signi-

Table 1. Comparison of clinical characteristics between groups 1 and 2

Characteristic	Group 1 (n=52)	Group 2 (n=39)	*P* value
Age (mo)	30.9±20.7	37.1±20.2	0.159
Fever duration at initial treatment (day)	5.7±1.1	5.5±1.1	0.461
Sex			0.009
Male	38 (73.1)	18 (46.2)	
Female	14 (26.9)	21 (53.8)	
Clinical manifestations			
Polymorphic rash	35 (67.3)	35 (89.7)	0.012
Erythema of the lips and oral mucosa	48 (92.3)	38 (97.4)	0.427
Cervical lymphadenopathy	32 (61.5)	20 (51.3)	0.328
BCG scar redness and erythema	19 (36.5)	15 (38.5)	0.851
Bilateral nonexudative conjunctivitis	49 (94.2)	35 (89.7)	0.427
Change of the extremities	46 (88.5)	31 (79.5)	0.240
Pyuria	10 (19.2)	11 (28.2)	0.315

Values are presented as mean±standard deviation or number (%).
Group 1, normal vitamin D group; group 2, vitamin D deficiency group; BCG, Bacille de Calmette-Guerin vaccine.

Table 2. Comparison of laboratory data between groups 1 and 2

Variable	Group 1 (n=52)	Group 2 (n=39)	*P* value
WBC ($\times10^3$/mm^3)	12.7±4.3	14.9±6.3	0.06
Neutrophil count ($\times10^3$/mm^3)	8.4±4.3	10.8±6.8	0.059
Neutrophil count (%)	63.7±15.5	68.4±17.3	0.178
Hemoglobin (g/dL)	11.6±0.9	11.2±1.2	0.107
Hematocrit (%)	33.0±2.3	32.4±3.2	0.301
Platelet ($\times10^3$/mm^3)	343.4±102.9	319.7±77.3	0.230
ESR (mm/hr)	26.8±17.8	31.1±22.6	0.326
CRP (mg/dL)	4.8±5.7	7.1±6.5	0.074
AST (U/L)	62.4±88.6	102.2±163.5	0.174
ALT (U/L)	69.90±109.65	97.2±124.1	0.270
Calcium (mg/dL)	9.5±0.5	9.06±0.49	<0.001
Phosphate (mg/dL)	4.3±0.9	3.9±0.7	0.007
Sodium (mEq/L)	137.6±2.9	136.3±2.7	0.03
Potassium (mEq/L)	4.6±0.6	4.4±0.5	0.068
ALP (IU/L)	198.6±79.7	206.5±87.2	0.664
NT-proBNP (pg/mL)	697.3±1,218.9	804.1±1,273.1	0.686
Albumin (g/dL)	3.8±0.3	3.6±0.4	0.028
Protein (g/dL)	6.6±0.6	6.5±0.7	0.894
Total bilirubin (mg/dL)	0.5±0.4	0.6±0.7	0.303
25-OH-vitamin D (ng/mL)	27.5±6.4	12.8±5.1	<0.001

Values are presented as mean±standard deviation.
Group 1, normal vitamin D group; group 2, vitamin D deficiency group; WBC, white blood cell; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; NT-proBNP, N-terminal fragment of B-type natriuretic peptide.

ficant difference in the incidence of lip and oral lesions, cervical lymphadenopathy, change in extremities, BCG scar redness and erythema, and bilateral nonexudative conjunctivitis between the 2 groups. In contrast, the incidence of polymorphic rash was significantly higher (*P*=0.012) in group 2 (89.7%) than in group 1 (67.0%). All data on clinical characteristics are summarized in Table 1.

Regarding the blood test results, group 1 had significantly higher levels of calcium (*P*<0.001), phosphate (*P*=0.007), sodium (*P*=0.03), and albumin (*P*=0.028) than group 2 did. In contrast, there were no significant differences between the 2 groups in leukocyte and neutrophil counts, neutrophil percent, platelet count, hemoglobin, AST, ALT, ALP, NT-proBNP, protein, total bilirubin, and CRP levels, as well as the hematocrit and ESR (Table 2). However, based on the echocardiographic findings, the LMCA *z* score was calculated at 1.67 ±0.65 in group 1 and 1.90±0.79 in group 2, while the RCA *z* scores for groups 1 and 2 were 1.42±0.66 and 1.33±0.80, respectively. The differences in either LMCA or RCA score between the 2 groups were not statistically significant (Table 3; LMCA score, *P*=0.126; RCA score, *P*=0.547). Finally, the crossover analysis, whose results are shown in Table 4, revealed that 4 patients (7.7%) in group 1 and 7 patients (17.9%) in group 2 exhibited coronary artery complications but this difference was not statistically significant, while there was

Table 3. Comparison of *z* score between groups 1 and 2

Variable	Group 1 (n=52)	Group 2 (n=39)	*P* value
LMCA diameters (mm)	2.74±0.36	2.73±0.38	0.876
LMCA *z* score	1.67±0.65	1.90±0.79	0.126
RCA diameters (mm)	2.38±0.33	2.27±0.38	0.149
RCA z score	1.42±0.66	1.33±0.80	0.547

Values are presented as mean±standard deviation.
Group 1, normal vitamin D group; group 2, vitamin D deficiency group; LMCA, left main coronary artery; RCA, right coronary artery.

Table 4. Comparison of characteristics between groups 1 and 2

Variable	Group 1 (n=52)	Group 2 (n=39)	*P* value
Coronary artery complication	4 (7.7)	7 (17.9)	0.137
IVIG resistance	6 (11.5)	12 (30.8)	0.023

Values are presented as number (%).
Group 1, normal vitamin D group; group 2, vitamin D deficiency group; IVIG, intravenous immunoglobulin.

a significant difference (*P*=0.023) in immunoglobulin resistance between group 1 (n=6, 11.5%) and group 2 (n=12, 30.8%).

Discussion

KD is an acute vasculitis of unknown etiology, in which the presence of coronary artery complications is important for prognosis. In the early acute phase, the plasma levels of cytokines such as interferon-γ, interleukin (IL)-4 and IL-10 are significantly higher than in the convalescent phase[13]. The inflammatory response progresses from the inside to the outside of the vessel during this phase. Neutrophils flood into the vessel, and are followed by monocytes and IgA plasma cells, which damage the blood vessels, resulting in complications such as coronary artery dilatation. The remodeling process, in which metalloproteinase activity is involved, is completed when the damaged blood vessels undergo fibrogenesis[10]. Immunoglobulin-resistant KD has been reported to be associated with a higher incidence of coronary artery complications[14,15]. As IVIG resistance and related risk factors have been widely reported to affect KD treatment, several studies attempted to find markers for predicting immunoglobulin-resistant KD[15-17]. Recently, vitamin D has been the focus of a large number of published studies. Vitamin D has been known to affect various diseases. For example, it lowers blood pressure[18], and a low vitamin D level increases not only the incidence of cardiovascular disease but also the associated mortality[19,20]. In addition, vitamin D exerts an anti-inflammatory effect by increasing IL-10 and inhibiting metalloproteinase synthesis, platelet aggregation, and prostaglandin production. As the anti-inflammatory activity of vitamin D allows it to inhibit the development of cardiovascular disease[21], we decided to focus on this activity and investigate the relationship between vitamin D levels and KD.

In the normal vitamin D level group, the male to female ratio was

significantly higher than in the vitamin D deficiency group. This result is consistent with a previous study according to which vitamin D levels are determined by sex[22]. Another study indicated that the prevalence of vitamin D deficiency in Koreans was higher in girls but found no age-dependent differences in the normal vitamin D levels[23].

The clinical manifestations of KD were not significantly different between the 2 groups, except for polymorphic rash incidence. Although, we do not yet fully understand the pathogenesis of KD, vascular endothelial growth factor (VEGF) might be a important role in KD clinical manifestation. VEGF is a vascular permeability factor that exerts angiogenic effects on targeted vascular endothelial cells. Increased VEGF levels have been reported in KD patients developing skin rash or edema of hands and feet[24]. Vitamin D has been reported to inhibit VEGF expression in various human cells under hypoxic conditions[25]. One study showed that vitamin D supplementation decreased serum VEGF levels and improved the clinical symptoms in women with polycystic ovary syndrome[26]. This effect of vitamin D on VEGF levels might explain the higher incidence of polymorphic rash in the vitamin D deficiency group. The lower serum albumin level of the vitamin D deficiency group in our study can also be attributed to the effect of vitamin D on VEGF level, as VEGF might increase vascular permeability and cause hypoalbuminemia[24].

The serum levels of calcium, sodium, and phosphorus were significantly lower in the vitamin D deficiency group. The low levels of calcium and phosphate are thought to be the result of the vitamin D concentration difference. The association between vitamin D deficiency and the low levels of calcium and phosphate can be explained by the role of vitamin D in their homeostasis.

During the acute phase of KD, the levels of inflammatory cytokine such as IL-6 and IL-1β are elevated. As these cytokines are related with antidiuretic hormone secretion, it has been suggested that they may be involved with the incidence of hyponatremia in KD[27]. Thus, these effects of vitamin D may explain the lower serum sodium level in the vitamin D deficiency group that was observed in the current study. NT-pro BNP was not significantly different between the 2 groups. This result is similar to that of a previous study on children[28]. As was previously mentioned, vitamin D exerts its anti-inflammatory activity through several ways and its supplementation reduced inflammatory marker including IL-6 and CRP[3]. Another study[29] reported that low vitamin D levels increase coronary artery complications in KD and serum vitamin D concentration is negatively associated with ESR and CRP. However we did not observe any differences in the incidence of coronary artery complications, ESR, or CRP between the 2 groups. These inconsistencies might be due to our small sample size and the fact that we did not include incomplete KD cases in our study.

The proportion of patients displaying resistance to immunoglobulin therapy was significantly higher in the vitamin D deficiency group. Although the effects of vitamin D on KD have not been elucidated, based on the reported effects of vitamin D on various disease, we suspect this is result of the anti-inflammatory effect of vitamin D. Although, we could not establish vitamin D as a significant predictor of resistance to immunoglobulin therapy, our study showed that vitamin D may have a positive effect on the prevention and treatment of KD.

This study had several limitations. First, the number of enrolled patients was relatively small. Second, this study did not consider the seasonal variations[23] that vitamin D levels exhibit.

In summary, we studied patients diagnosed with KD and showed that proportion of resistance to immunoglobulin therapy and polymorphic rash were higher in the group with vitamin D level lower than 20 ng/mL, whereas the same group had lower levels of sodium and albumin. These results might be due to the anti-inflammatory effects of vitamin D. Thus, low vitamin D levels might be associated with resistance to the IVIG therapy in KD. We have shown the potential of vitamin D to play an important role in the prevention and treatment in KD.

There are already many studies on the severity and treatment of KD that take the important implications of inflammation in KD pathophysiology into account.

Further studies on the effect that vitamin D exerts on KD via its anti-inflammatory activity are needed.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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