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Serum 25-hydroxy Vitamin D in Japanese Children with PFAPA Syndrome

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Abstract

Objectives: Low levels of blood vitamin D have been reported not only in children with otitis media and frequent respiratory tract infections, but also in PFAPA syndrome patients. Levels of vitamin D are influenced regional and racial differences.

Methods and Results: We measured the concentrations of 25-OH vitamin D in the serum of Japanese patients with PFAPA. Serum levels of 25-OH vitamin D of the 14 subjects were 13.7 to 29.0 ng/mL, Five out of 14 subjects (35.7%) had levels less than 20 ng/mL, which is assessed as vitamin D deficiency. There was a significant difference in serum vitamin D levels between the PFAPA syndrome group and the age-matched control group (P = 0.03). The levels of vitamin D of 16 PFAPA patients, 8 subjects with intractable otitis media and 11 healthy controls were measured. Serum levels of patients with PFAPA syndrome and intractable otitis media were significantly lower than those of controls. There was no statistically significant difference in the levels of 25-OH vitamin D with sex, fever, duration of the febrile period, serum level of amyloid A, and frequency of occurrence of aphthous stomatitis. However, the occurrence of aphthous stomatitis was more frequent in patients with low levels of 25-OH vitamin D compared with patients with higher levels. There was no specific polymorphism compared with controls and past genomic data in 6 candidates of SNPs of *VDR*.

Conclusions: Serum levels of Japanese patients with PFAPA syndrome were significantly lower than those of controls. Vitamin D might correlate with symptoms of PFAPA.

Keywords: Vitamin D deficiency; Cytokine; T-cell; *VDR*; 1,25-$(OH)_2$ vitamin D3

Introduction

The pathogenesis of periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome is still unknown, and antipyretics and antibiotics are not effective in controlling the fever attacks. The administration of histamine-2 receptor antagonists (H2 blockers) or colchicine as a prophylactic therapy, oral steroids as a symptomatic therapy, and tonsillectomy for possible curative therapy have been performed to control the symptoms [1]. In recent years, the effects of vitamin D on immunity have been attracting attention. Low levels of blood vitamin D have been reported not only in children with otitis media and frequent respiratory tract infections but also in PFAPA syndrome

patients [2,3]. Vitamin D levels are different depending on regions due to the duration of sunshine. In this study, we measured the concentrations of 25-hydroxy vitamin D (25-OH vitamin D) to elucidate the involvement of vitamin D in Japanese pediatric patients with PFAPA. In addition, we also investigated whether *VDR* polymorphisms were correlated with PFAPA.

Subjects and methods

To know the association between vitamin D and PFAPA syndrome, 14 patients (6 boys and 8 girls) aged 0 to 9-years old who were followed at the Department of Pediatrics, Tokyo Medical University Hospital, from January 2014 to August 2018 were enrolled in this study. All patients were diagnosed as having PFAPA syndrome by Thomas criteria [4]. All samples except one were collected in the spring or summer. The levels of serum 25- OH vitamin D were measured by the chemiluminescence immunoassay method and categorized into 3 levels, as follows: more than 30 ng/mL (normal range), 30–20 ng/mL (low level), and less than 20 ng/mL (deficient level). Statistical analyses were performed by the *t*-test.

We also investigated polymorphisms of *VDR*, which has been reported to be correlated with susceptibility to infectious agent Laplana M [5] by using exome data of them. Exome sequencing was performed with the Ion Proton sequencer (Thermo Fisher Scientific).

Results

Serum levels of 25-OH vitamin D of the 14 subjects were 13.7 to 29.0 ng/mL. Five out of 14 subjects (35.7%) had levels less than 20 ng/mL, which is defined as vitamin D deficiency. There was a significant difference in serum vitamin D levels between the PFAPA syndrome group (mean 21.4 ng/mL) and the age-matched control group (28.5 ng/mL) ($P = 0.03$) (Figure 1).

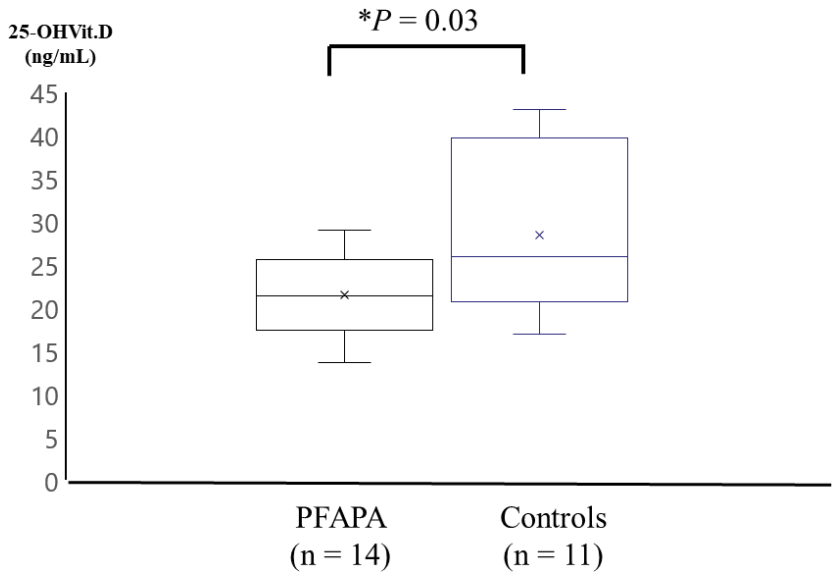

Figure 1: Serum levels of 25-OH vitamin D in patients with PFAPA and controls (* means statistically significant).

The levels of vitamin D of 8 other patients (8 subjects with intractable otitis media) and 11 healthy controls are shown in figure 2. Serum 25-OH vitamin D levels of patients with intractable otitis media and PFAPA were significantly lower than those of controls. There was neither significant difference between the febrile group and the afebrile group regarding 25-OH vitamin D levels (Figures 2 & 3), nor statistically significant difference with sex, duration of the febrile period, serum level of amyloid A, and frequency of occurrence of aphthous stomatitis (Table 1). However, the occurrence of aphthous stomatitis was more frequent in patients with low levels of 25-OH vitamin D compared with patients with higher levels. Polymorphism of *VDR* did not show any significant difference between patients with PFAPA and in-house or Asian data, which were shown in Table 2.

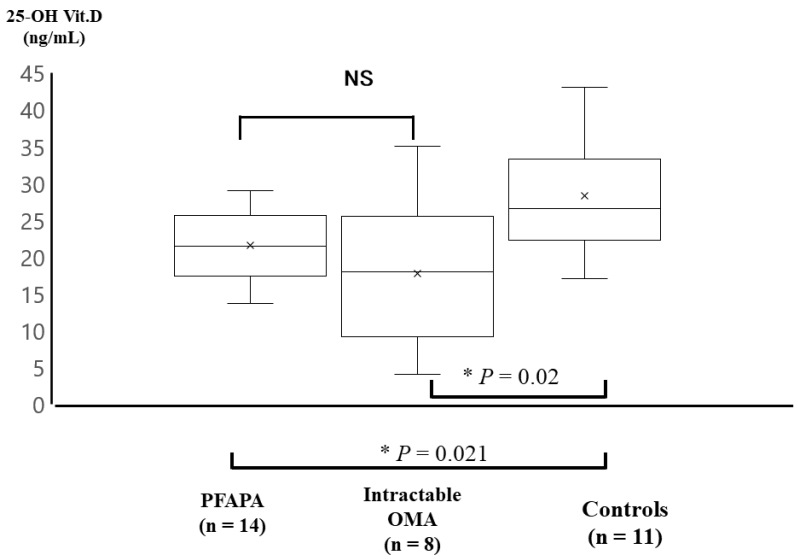

Figure 2: Serum levels of 25-OH vitamin D in patients with PFAPA and frequent otitis media (* statistically significant, OMA: Otitis Media Acute, NS means no statistically significant).

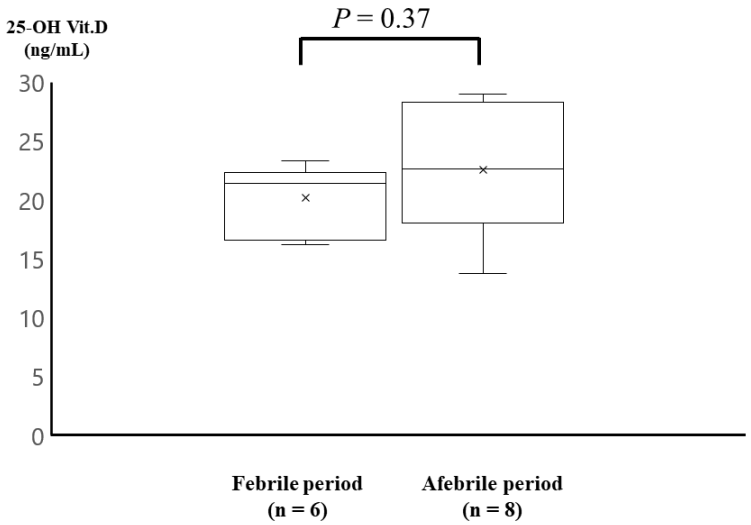

Figure 3: Serum levels of 25-OH vitamin D in patients with PFAPA in both the febrile and afebrile period: There was no statistically significant (P=0.37).

25-OH vitamin D (ng/mL)	n	Age (months) mean ± SD	Sex (M/F)	Duration of febrile period (weeks)	SAA* max	Aphthous stomatitis (case/total cases)	Treatments
< 20	5	41 ± 24	M:2 F:3	2-3	9.9-1070	4/5 (80%)	H_2 blocker Colchicine Tonsillectomy
20-30	9	60 ± 35	M:4 F:5	2.8-3.8	2.5-760	3/9 (33%)	H_2 blocker Colchicine

*SAA: Serum amyloid A

Table 1: Patient profiles and symptoms in different categories of 25-OH vitamin D levels

poly morphism (rs code)	alleles	Global	HGVD (reference / alternative)	ToMMO (reference / alternative)	in house (75 samples) (reference/alternative)	case 1	case 2	case 3	case 4	case 5	case 6	case 7	case 8
Cdx (rs11568820)	C>T	0.631/ 0.3639	NA/NA	0.6008/0.3992	NA	CC	CC	CC	CC	CC	CC	CC	CC
A1021 (rs4516035)	T>C	0.6815/ 0.3185	0.987/0.013	0.9843/0.0157	NA	TT	TT	TT	TT	TT	TT	TT	TT
FokI (rs2228570)	A>C,G,T	0.37050/ 0.62950	0.381/0.619	0.3673/0.6326	0.29/0.71	GG	AG	AA	AG	GG	GG	AA	AG
Bsm I (rs1544410)	C>A,G,T	0.66538/ 0.33462	0.9/0.100	NA	NA	CC	CC	CC	CC	CC	CC	CC	CC
ApaI (rs7975232)	C>A	0.48784/ 0.51216	0.656/0.344	0.6789/NA	NA	CC	CC	CC	CC	CC	CC	CC	CC
TaqI (rs731236)	A>G	0.67164/ 0.32836	0.905/0.095	0.8949/-	0.88/0.12	AA	AA	AA	AA	AA	AA	AA	AA

NA means "not available".

Table 2: Allele of VDR polymorphisms in 8 cases with PFAPA (data of in house were used excluding immunological disorders).

Discussion

Vitamin D is associated with bone remodeling, and also plays roles in enhancing immune function and reducing inflammation. Low levels of 1,25-$(OH)_2$ vitamin D are associated with the suppression of inflammatory cytokines, production of antimicrobial peptides, and production of IgG [6]. A vitamin D-deficient state has been reported to be associated with the frequency and severity of otitis media [7,8]. Recently, patients with PFAPA syndrome have been reported to be deficient in vitamin D. Mahamid [2] and Stagi [3] reported that blood vitamin D levels were significantly lower in PFAPA patients than in controls [2,3]. Our results of Japanese PFAPA children are consistent with the results of these previous reports. Generally, vitamin D levels are significantly lower in the winter than in the summer [6,9]. In our present study, blood samples of 13 out of the 14 patients with PFAPA were collected in the spring to summer; nevertheless, vitamin D levels were lower in the majority of our PFAPA patients than in the controls. Therefore, the low levels of vitamin D observed in patients with PFAPA appear to be a consistent result observed throughout all seasons. The pathophysiology of PFAPA has remained unclear to date. Vitamin D deficiency has been suggested to be correlated with the onset of PFAPA. Nalbantoğlu [10] reported that C-reactive protein and serum 25-OH vitamin D levels of less than 30 ng/mL were associated with the onset of PFAPA [10]. Vitamin D supplementation has been shown to decrease fever durations and to prolong fever-free periods in patients with PFAPA [3]. The fact that the symptoms of most children improve with age also supports this hypothesis.

Alternatively, vitamin D deficiency might occur secondarily to immunological status. In our study, none of the patients experienced a disappearance of their symptoms by vitamin D supplementation, although 1 patient demonstrated an extension of the fever- free period after the start of vitamin D administration. Stojanov [11] analyzed serum and intracellular cytokine levels in 6 patients with PFAPA, and reported that febrile PFAPA attacks led to a significant increase in serum levels of IL(Interleukin)-6 and IFN (Interferon)-γ compared with symptom-free periods and controls, accompanied with higher levels of IL-1β, tumor necrosis factor-α, and IL-12 (p70) than in controls [10]. In addition, they reported that serum levels of the anti-inflammatory cytokine IL-4 were lower in PFAPA patients than in controls at all times analyzed [11]. Forsvoll [6] reported that activation of the innate immune response is the initial step in PFAPA, followed by an adaptive response with the activation and redistribution of T-cells [12]. They found that levels of IL-6, CXCL10, and CCL4 were significantly increased during febrile episodes, whereas the level of CXCL10 remained high also between febrile episodes. Their results showing inflammatory cytokine activation and a reduced anti-inflammatory response suggest a dysregulation of the immune response in patients with PFAPA syndrome. FDG-PET/CT imaging of a patient with PFAPA displayed increased FDG uptake in the bones or bone marrow throughout the entire body (figure 4). Therefore, bone metabolism may be increased in patients with PFAPA, and consequently affect relative 25-OH vitamin D deficiency.

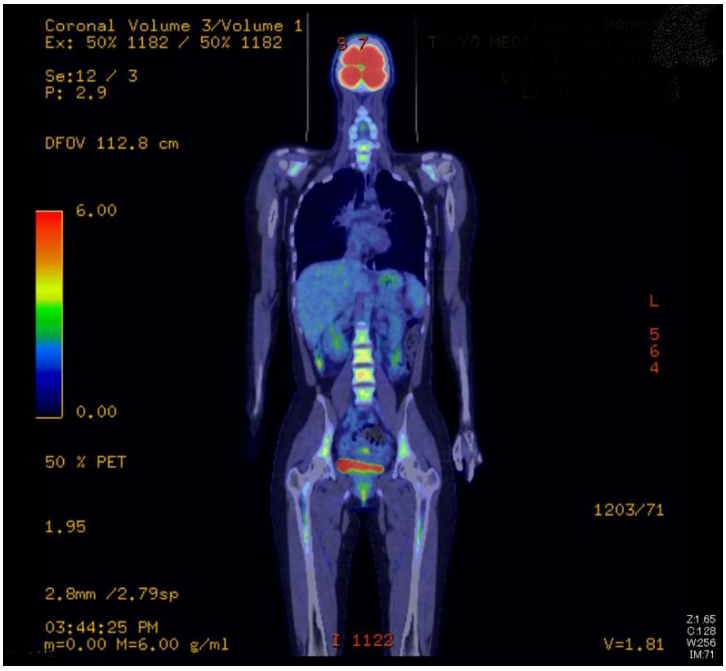

Figure 4: FDG-PET/CT imaging obtained from 20 years-old girl with PFAPA. The imaging displayed clearly increased FDG uptake in the bone marrow throughout the entire body.

Rausch Fan [13] analyzed the effects of 1,25-$(OH)_2$ vitamin D3 (the active form of vitamin D) on cytokine secretion in human peripheral blood mononuclear cells (PBMCs), and allergen-specific T-helper (Th) cell clones. The production of IL-2, IFN-γ, and IL-12 by PBMCs and Th1 clones was significantly inhibited by 1,25-$(OH)_2$ vitamin D3. The treatment of PBMCs with 1,25-$(OH)_2$ vitamin D3 induced the production of IL-10, which is a representative suppressive cytokine, but not the production of IL-4 [13]. These findings demonstrate that low levels of 25-OH vitamin D in children with PFAPA syndrome are compatible with their cyclic fever and fluctuations in cytokine levels. Therefore, a sufficient supply of 1,25-$(OH)_2$ vitamin D3 may be useful as an immunomodulation therapy.

The actions of vitamin D are mediated by its receptor (VDR), which acts as a ligand- dependent transcription factor and plays a pivotal role in immune response modulation. Therefore, *VDR* polymorphisms were suspected to be associated with PFAPA. However, none of our present subjects had any of the 6 candidate SNPs (Single-nucleotide polymorphisms) identified in previous genomic analyses of PFAPA-associated SNPs.

The limitations of this study involve seasonable bias and were based on medical records, and hence it is necessary to conduct further studies to clarify potential confounding factors and the effectiveness of vitamin D supplementation for controlling PFAPA symptoms.

Author contribution

J.S. and HK. designed the study; K.K., S.S., S.M., and Y.K. performed experiments; K.K, collected and analyzed data; J.S.,T.Y., and HK. wrote the manuscript; G.Y. gave technical support and conceptual advice. All authors read and approved the final manuscript. T.Y. critically reviewed the manuscript and supervised the whole study process. All authors read and approved the final manuscript.

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