

Vitamin D Deficiency in the Absence of Enteropathy in Three Cases with Common Variable Immunodeficiency

Ömür Ardeniz^a Çıgır Biray Avcı^c Aytul Sin^a Gökhan Özgen^b Fulya Günsar^d Nihal Mete^a
Okan Gulbahar^a Ali Kokuludag^a

^aDivision of Allergy and Clinical Immunology and ^bDivision of Endocrinology, Department of Internal Medicine,

^cDepartment of Medical Biology and ^dDepartment of Gastroenterology, Ege University Medical Faculty, Izmir, Turkey

Established Facts

- Common variable immunodeficiency (CVID) is characterized by recurrent infections and defective antibody production.
- Development of granulomatous disease, tendency to autoimmune diseases and malignancies in particular B cell lymphoma are the other well-known properties of CVID.
- These patients can also develop malabsorption due to an idiopathic inflammatory bowel disease leading to chronic diarrhea and nutrient deficiencies.

Novel Insights

- Herein we reported 3 patients with CVID in whom vitamin D deficiency was detected in the absence of clinical findings suggestive of a malabsorption state. Furthermore, none of the patients presented nutrient deficiencies other than vitamin D.
- Vitamin D receptor expression was also found to be lower in peripheral blood mononuclear cells and hair follicles of these patients compared to the control group.
- These findings would be shared by a subgroup of patients with CVID and altered vitamin D metabolism could be one of the reasons of the increased tendency to inflammation, autoimmunity and malignancy.
- Patients with CVID may present asymptomatic vitamin D deficiency in the absence of enteropathy. Persistently high alkaline phosphatase levels may be the only remarkable sign.

Key Words

Immunodeficiency • Immunomodulation • Vitamin D₃ •
Common variable immunodeficiency • Vitamin D
deficiency • Vitamin D receptor expression

Abstract

Background: Common variable immunodeficiency (CVID) is characterized by hypogammaglobulinemia and a defect in

antibody production. Herein we describe 3 patients diagnosed with CVID in whom vitamin D deficiency was detected in the absence of enteropathy. **Methods:** Biochemical and immunological analysis, serum osteocalcin, parathyroid hormone, 25-OH vitamin D, 1,25(OH)₂ vitamin D, vitamin A, vitamin E, urinary calcium, and deoxypyridinoline measurements were carried out. Vitamin D receptor (VDR) expression was examined in the peripheral blood mononuclear cells and hair follicles by reverse transcriptase polymerase chain

reaction. VDR gene polymorphism was evaluated by high-performance liquid chromatography. **Results:** None of the patients presented nutrient deficiencies other than vitamin D. Two of them were free of osteomalacia-related symptoms. VDR expression was found to be lower in the peripheral blood mononuclear cells and hair follicles when compared to the control group. **Conclusions:** Patients with CVID may present asymptomatic vitamin D deficiency. Vitamin D and VDRs play an important role in the innate immune system and modulate Toll-like receptor-related responses. Delay in diagnosis may predispose these patients not only to irreparable bone loss but also to infections, and autoimmune and malignant disorders, thus emphasizing the importance of prompt intervention.

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Introduction

Common variable immunodeficiency (CVID) is the most prevalent primary immunodeficiency characterized by recurrent infections and defective antibody production [1]. Herein we describe 3 patients diagnosed with CVID in whom significant vitamin D (vit D) deficiency was detected in the absence of an overt underlying abnormality.

One of these patients presented giardiasis and nodular lymphoid hyperplasia in the absence of malabsorption-related symptoms. Fecal fat excretion was found to be increased; nevertheless, other fat-soluble vitamin levels were reported to be normal. Interestingly, none of the patients presented nutrient deficiencies other than vit D. Two of them were free of osteomalacia-related symptoms.

Although these patients had different clinical presentations, vitamin D receptor (VDR) expression was found to be lower in the peripheral blood mononuclear cells (PBMC) and hair follicles (HF) when compared to the control. It has been shown that VDRs play an important role in the innate immune system and modulate Toll-like receptor (TLR)-related responses [2]. TLR dysfunction leading to defective interferon- α production and reduced antibody synthesis has been demonstrated in patients with CVID [3]. In the light of these findings, abnormal vit D metabolism and reduced VDR expression in the immune effector cells appear to contribute to the dysfunction of the acquired immune system in a subgroup of patients with CVID. Additionally, reduced VDR expression would play a role in inflammation and granuloma formation.

We also wanted to draw attention to the fact that patients with CVID may present with osteomalacia in the absence of enteropathy. As they may not complain of osteomalacia-related symptoms, it can be missed without further investigation and high alkaline phosphatase (ALP) levels may be the only remarkable sign.

Method

Relative Quantification of VDR

Two milliliters of blood and 10 hair follicles were collected from each case. Fifty microliters of total RNA was isolated from 200 μ l PBMC and HF by using High Pure RNA Isolation Kit (Roche, Germany). The amount of total RNA used for reverse transcription was in the range of 1–5 μ g and OD260/280 in the interval of 1.9–2.1. Ten microliters of total RNA was used for reverse transcription to cDNA and 20 μ l of cDNA was obtained. Reverse transcription procedure was performed for cDNA synthesis by using Transcriptor First Strand cDNA Synthesis Kit according to the manufacturer's instructions. The control group included 8 healthy females with an age range of 20–34 years.

Real-time quantitative RT-PCR analyses of VDR were performed with Lightcycler instrument and software. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH* 'housekeeping' gene) was chosen as an internal standard to control for variability in amplification. The sequences of primers and probes used are shown in table 1. PCR was performed by using a TaqMan Master Kit (Roche Diagnostics, Germany) according to the instructions of the manufacturer. The VDR target probe was labeled at the 5' end with the reporter dye molecule 6-carboxyfluorescein (FAM). The *GAPDH* target probe was labeled with 6-carboxyfluorescein. Both probes were labeled with the quencher fluor 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. To quantify VDR mRNA from PBMC and HF, we constructed a calibration curve (error: 0.100; efficiency: 1,790) using *GAPDH* mRNA as an endogenous control.

Analysis of the VDR Gene Polymorphism

DNA was extracted from venous blood using Magnesil DNA Isolation Kit (MD 1460, Promega, USA). Following PCR reactions all four single-nucleotide polymorphisms (SNPs) *FokI*, *BsmI*, *ApaI* and *TaqI* within the VDR gene were genotyped with denaturing high-performance liquid chromatography. Primers, PCR annealing temperatures and denaturing high-performance liquid chromatography melting temperatures are listed in table 2. With this method homozygous alleles produce a single peak and heterozygous alleles produce two peaks (fig. 1).

Case 1

A 23-year-old female diagnosed as having CVID in 1999 was admitted to hospital after an evaluation of her general status in November 2005.

Her medical history revealed 6–8 hospitalizations/year caused by airway tract infections and diarrhea since the age of 11 years with an increased frequency after 17 years of age. She was given a diagnosis of bronchiectasia in 1999 and further evaluation revealed low levels of all immunoglobulin types: IgG 225 mg/dl

Fig. 1. Homoduplex (a) and heteroduplex (b) peaks of *FokI* polymorphism.

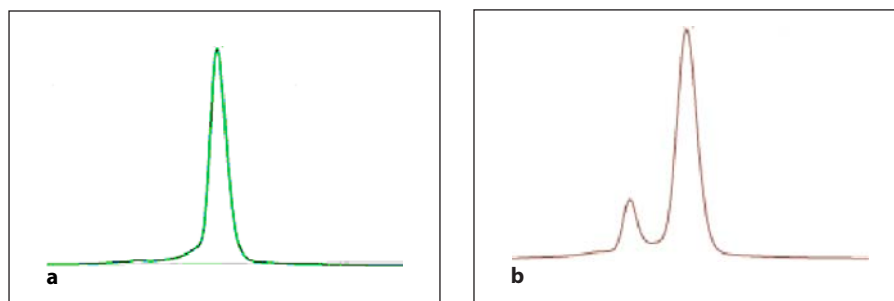


Table 1. VDR mRNA expression and GAPDH primer/probe sequence 5'–3'

VDR probe	FAM-CCT GCC GGC TCA AAC GCT GTG-TAMRA
VDR forward primer	CTC ATC TGT CAG AAT GAA CTC CTT
VDR reverse primer	TCA CCA AGG ACA ACC GAC G
GAPDH probe	FAM- <i>caa gct tcc cgt tct cag cc</i> -TAMRA
GAPDH forward primer	<i>gaa ggt gaa ggt cgg agt c</i>
GAPDH reverse primer	<i>gaa gat ggt gat ggg att tc</i>

Table 2. List of the primers, annealing temperatures and DHPLC melting temperatures

VDR polymorphism	Primers	PCR annealing temperature °C	DHPLC melting temperature °C
<i>FokI</i>	F: CCCTGGCACTGACTCTGCTC R: GGAAACACCTTGCTTCTTCTCC	60	61.9
<i>BsmI</i>	F: AGTGTGCAGGCGATTTCGTAG R: ATAGGCAGAACCATCTCTCAG	60	60.8
<i>ApaI</i>	F: AGCATGGACAGGGAGCAA R: CCTGTGCCTTCTTCTCTATCC	61.9	61.9
<i>TaqI</i>	F: CCTGTGCCTTCTTCTCTATCC R: AGCCTGAGTGCAGCAGTA	54	61.6

DHPLC = Denaturing high-performance liquid chromatography.

(normal: 700–1,600), IgA <24 mg/dl (normal: 70–400), and IgM <16 mg/dl (normal: 40–230). Anti-*Haemophilus* antibody titer after vaccination was 0.118 µg/ml [basal value was 0.089 (normal: >1)] and antitetanus antibody titer was found to be <0.01 IU/ml (normal: >0.1). After the patient had been started on intravenous immunoglobulin therapy, a lobectomy was performed in 2000.

There was no family history of consanguinity. She was hospitalized because of upper and lower respiratory tract infections 2–3 times/year after having been put on intravenous immunoglobulin therapy. She had never experienced life-threatening infections caused by opportunistic pathogens, viruses or fungi by this time. She was also diagnosed with type 1 diabetes mellitus and has been on insulin therapy since 2000. In 2003, the patient

was also diagnosed as having polyneuropathy and significant improvement was observed with gabapentin therapy. Within a short period of time after the diagnosis of polyneuropathy, sensorineural hearing loss developed. It was considered whether it had an autoimmune origin because of its rapid progression and absence of any contributing factor such as infection, trauma from exposure to a loud sound or drug use. However, spontaneous improvement without any immunosuppressive medication raised questions about the exact mechanism.

On admission, she did not report diarrhea or weight loss. The physical examination was normal except for reduced sounds in the left lower part of the lung, which was associated with lobectomy. Her vital signs were as follows: temperature 36.5°C, pulse

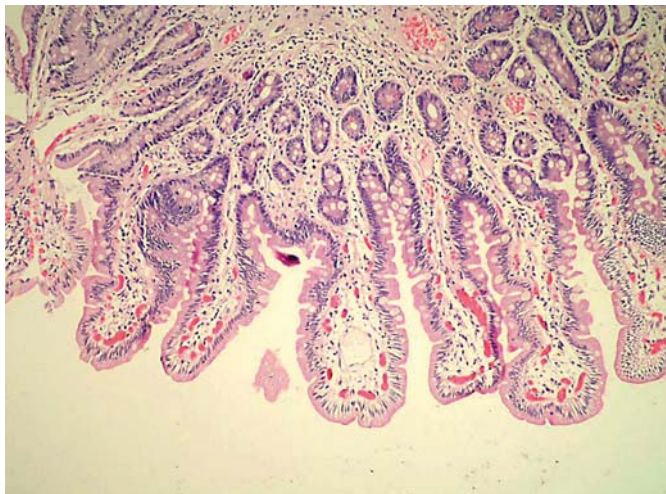


Fig. 2. Case 1. Slightly congested duodenal mucosa. Villous architecture was well-preserved. HE. $\times 10$.

90 beats/min, and blood pressure 110/80 mm Hg. Her weight was 52 kg and her body mass index (BMI) 19.33. She was active when she was not suffering from infections.

Quantitative immunoglobulin evaluation revealed hypogammaglobulinemia (table 3). Absolute numbers of the peripheral blood lymphocyte subsets by flow cytometry (Becton Dickinson) on admission are shown in table 3.

Laboratory analysis revealed Hct 37%, leukocytes 10,000/ μ l, lymphocytes 1,500/ μ l, monocytes 100/ μ l, and platelets 178,000/ μ l. The biochemical analysis revealed an isolated high ALP level (table 4), which had been a persistent finding since 1999. Other parameters including sedimentation rate, C-reactive protein and thyroid function tests were in the normal range (table 4). Autoantibodies including antinuclear (ANA), antimitochondrial (AMA), AMA-M2, anti-smooth muscle (ASMA), liver-kidney microsomal (LKM-1), gastric parietal cell (GPC), antineutrophil cytoplasmic (ANCA), rheumatoid factor, antithyroid, antitransglutaminase and antigliadin antibodies were found to be negative. The high-resolution computed tomography (HRCT) of the thorax and CT of the abdomen did not suggest granulomatous disease.

The persistent isolated high ALP levels in the absence of granulomatous disease prompted us to search for an underlying bone metabolism abnormality. Serum level of osteocalcin was found to be increased consistent with increased bone turnover. Urinary deoxypyridinoline level, which is a reliable marker of bone resorption, was in the normal range as was the parathyroid hormone (PTH) level. The urinary calcium excretion was significantly reduced (table 5).

Osteomalacia and sometimes Paget's disease may be related to high osteocalcin levels [4]. The X-ray examination of the cranium and extremities revealed increased radiolucency. There were no specific abnormalities suggestive of Paget's disease. The bone density evaluation showed osteoporosis. The T score for the left hip was 3.2 and for the lumbar spine it was 3.00. The bone radionuclide scan was found to be normal as were the blood calcium, phosphate and magnesium levels (table 4). Although vit A and E levels were

Table 3. Lymphocyte subset analyses by flow cytometry and immunoglobulin levels of the 3 patients on admission

	Patient 1	Patient 2	Patient 3
CD3+ T cells/ μ l	1,400	1,260	6,300
CD19+ B cells/ μ l	100	90	500
CD4+T cells/ μ l	540	420	1,400
CD8+ T cells/ μ l	860	840	4,900
CD16+56+ NK cells/ μ l	4	150	200
IgG (700–1,400), mg/dl	660	863	625
IgA (70–400), mg/dl	<20	<20	<20
IgM (40–230), mg/dl	<15	<15	<15

Table 4. Biochemical parameters of the patients on admission

Values	Patient 1	Patient 2	Patient 3	Normal range
Sedimentation, mm/h	13	9	2	<15
C-reactive protein, mg/l	0.3	1.22	6	0–5
SGOT, U/l	15	35	46	5–35
SGPT, U/l	16	23	70	5–40
ALP, U/l	508	357	1,664	90–260
GGT, U/l	8	62	726	7–32
Direct bilirubin, mg/dl	0.2	0.1	0.1	0–0.25
Indirect bilirubin, mg/dl	0.71	0.3	0.2	0.1–0.9
Urea, mg/dl	18	19	19	10–50
Creatinine, mg/dl	0.5	0.65	0.6	0.6–1.1
Albumin, g/dl	4.1	4.9	4.9	3.5–5
Globulin, g/dl	2.2	3	2.2	2.5–3.5
Glucose, mg/dl	96	110	82	75–115
HbA1c, %	6	–	–	<6.5
Calcium, mg/dl	8.8	9.4	9.8	8.2–10.4
Phosphate, mg/dl	3.3	4.1	3.7	2.3–4.5
Magnesium, mg/dl	2.2	2.2	2.1	1.5–2.6
Vitamin B ₁₂ , pg/ml	243	481	620	197–866
Folate, ng/ml	8.16	13	6.64	3–16
Vitamin A, mg/l	1.7	0.81	1.1	0.3–1.1
Vitamin E, mg/l	8.4	8	9.2	6–18
Iron, μ g/dl	49	50	145	50–140
Ferritin, mg/ml	21.65	13.6	436	13–150
Hct, %	34.9	41.6	44.6	35–45

found to be normal (table 4), the 25-OH vit D and 1,25(OH)₂ vit D levels were lower than the normal range (table 5).

Despite the negative history of diarrhea and weight loss and absence of hypocalcemia or hypoalbuminemia associated with malabsorption, we searched for a gastrointestinal pathology that might be responsible for impaired vit D metabolism. Upper gastrointestinal tract endoscopy was performed and biopsies taken from the antrum and second part of the duodenum revealed normal mucosa. There was no increased number of intraepithelial

Table 5. Markers related to bone metabolism of case 1

Values	On admission	Treatment interruption	After treatment	Normal range
Osteocalcin, ng/ml	35.5	11	17	3.1–13.7
Deoxypyridinoline, nM/mM	6.3	–	6.7	3.0–7.4
Urinary calcium, mg/24 h	25	–	117	100–300
25-OH vit D, ng/ml	5	1.2	5	7.6–75
1,25(OH) ₂ vit D, pg/ml	<8	1.2	not performed	29.6–65.1
PTH, pg/ml	49	68	50.7	15–65
ALP, U/l	472	508	239	90–260

Table 6. VDR mRNA expression of the cases and the control

	Control				Cases				
	HF		PBMC		case No.	HF		PBMC	
	RR	total RNA μg/μl	RR	total RNA μg/μl		RR	total RNA μg/μl	RR	total RNA μg/μl
Expression	37.9825	0.482	0.1424	0.573	1	0.6907	0.300	0.0143	0.120
SD	18.9454		0.0130		2	0.0269	0.180	0.0003	0.682
95% CI	18.5661		0.0127		3	1.0640	0.206	0.0101	0.172

RR = Relative ratio.

lymphocytes suggesting celiac disease and giardiasis were not detected (fig. 2). There were no histiocytes in lamina propria suggestive of Whipple's disease. As she declined radiological examination of the small intestine, the amount of fecal fat was analyzed after a diet containing 100 g of fat/day and found to be normal (4.5 g/24 h, normal: <6 g).

We considered the patient's status as a preosteomalacic phase and calcitriol (Rocaltrol; Roche) 0.25 μg b.i.d. together with calcium (1 g/day) was started. Six months later, she was rehospitalized as a result of cessation of the therapy because of a lack of health insurance. 25-OH vit D and 1,25(OH)₂ vit D levels were found to be extremely low and the serum PTH level was found to be increased (table 5). Calcitriol 0.5 μg b.i.d. and calcium 1 g/day were started. Six months after starting this therapy, ALP had returned to normal and urinary calcium excretion was increased. Nevertheless, 25-OH vit D was still lower than normal (table 5).

VDR mRNA expression was analyzed in the PBMC and HF, and found to be decreased up to 10.04 and 1.82%, respectively, when compared with the control group (table 6). An analysis of the VDR gene polymorphism revealed that she was heterozygous for *Apal*, *TaqI* and *BsmI* but homozygous for the *FokI* gene (table 7).

At the end of 1 year of therapy, the 25-OH vit D level increased to the normal range (17.4 ng/ml, normal: 7.6–75).

Case 2

A 43-year-old female was given a diagnosis of CVID in 2002 after evaluation because of recurrent infections since childhood,

30 kg weight loss in 1 year, nausea, vomiting and diarrhea. The IgG level was 174 mg/dl (normal: 700–1,400) and IgA and IgM were undetectable at the time of diagnosis. Antitetanus antibody titer was reported to be 0.001 IU/ml (normal: >0.1) and anti-Haemophilus antibody titer was 0.08 μg/ml (normal: >1) after vaccination. Giardiasis was detected by duodenal biopsy performed by virtue of upper endoscopy and stool examination. Six months after the initiation of intravenous immunoglobulin and metronidazole therapy, her status stabilized and she reached normal weight. The patient was free of infections after having been put on immunoglobulin therapy. She did not report consanguinity, but her son was given the diagnosis of selective IgA deficiency.

The patient was hospitalized for a routine checkup in January 2007. She reported diarrhea (loose defecation once or twice a day) and dyspeptic symptoms 2 or 3 times a year without weight loss. Her vital signs were as follows: temperature 36.7°C, pulse 70 beats/min, blood pressure 100/60 mm Hg, and BMI 26. Physical examination was normal apart from hepatosplenomegaly.

Neither anemia nor hypoalbuminemia was detected (table 4). Hb was 12.7 g/dl, leukocytes 7,500/μl, lymphocytes 1,500/μl, neutrophil count 5,400/μl, and monocytes 600/μl. Autoantibodies were negative.

HRCT of the thorax did not show granulomatous disease. The abdominal CT revealed enlarged liver and spleen with homogeneous density and multiple mesenteric adenopathies. The calibration of the intestinal ansa was reported to be normal.

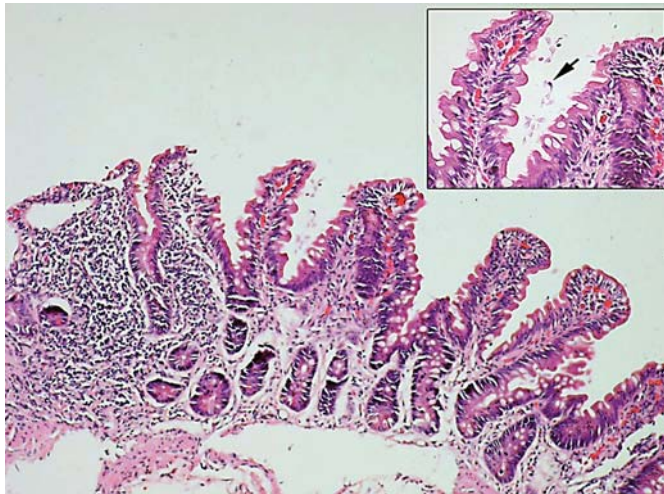


Fig. 3. Case 2. A lymphoid aggregate was present in the duodenal mucosa, but no intraepithelial lymphocyte was observed. Villous architecture was well-preserved. HE. $\times 10$. Note the Giardia trophozoites in the vicinity of villous tips (inset, arrow). HE. $\times 20$.

Table 7. VDR gene polymorphism evaluation of the three cases

VDR gene	Patient 1	Patient 2	Patient 3
<i>ApaI</i>	Aa	aa	Aa
<i>TaqI</i>	Tt	tt	Tt
<i>BsmI</i>	Bb	Bb	Bb
<i>FokI</i>	FF	ff	ff

Table 8. Markers related to bone metabolism of case 2

Values	On admission	After treatment	Normal range
Osteocalcin, ng/ml	35.1	11	3.1–13.7
Deoxypyridinoline, nM/mM	9	–	3–7.4
Urinary calcium, mg/24 h	26	127	100–300
25-OH vit D, ng/ml	6.5	–	10–40
1,25(OH) ₂ vit D, pg/ml	57	–	30–65
PTH, pg/ml	99	57	15–65
ALP, U/l	357	141	90–260

The upper endoscopic evaluation was performed and analysis of the biopsy specimen taken from the second part of the duodenum revealed giardiasis with mixed lymphoid infiltration (fig. 3). Stool examination confirmed the diagnosis of giardiasis and the patient was started on combined ornidazole and tetracycline

therapy. Stool examination was reported to be normal 2 weeks after the initiation of therapy and her diarrhea had ceased. Minimal focal fatty liver (1–2%) was detected in a liver biopsy without granuloma formation. Hepatitis serology turned out to be anti-HBs and anti-HBc-IgG positive and anti-HCV and HCV-RNA negative.

The bone mineral density evaluation was performed in view of the menopausal status of 1 year. T score for the left femur was found to be -2.2 , which is consistent with osteopenia and we decided to evaluate the bone metabolism of the patient. Thyroid function tests were normal. Serum osteocalcin and urinary deoxypyridinoline levels were found to be higher than normal (table 8). The radionuclide bone scan evaluation was normal as was the X-ray examination of the extremities. High PTH and low urinary calcium levels prompted us to evaluate the vit D status. The serum 25-OH vit D level was found to be lower than normal (table 8). VDR mRNA expression analysis in PBMC and HF revealed reduced levels compared with the control (table 6) and VDR gene polymorphism was analyzed (table 7).

Radiology of the small intestine was reported to be normal. Nevertheless, the amount of fecal fat excretion was found to be higher than normal (14 g/24 h). Colonoscopy was performed including the last 15 cm of the terminal ileum and found to be normal. The biopsy taken from the terminal ileum was reported to be normal.

Although oral therapy with calcitriol was initially introduced, we advised parenteral vit D therapy (300,000 IU/month) in view of the increased fecal fat excretion. However, the patient declined parenteral replacement and continued taking calcitriol 0.25 μ g b.i.d. together with calcium 1 g/day. Six months after starting this therapy, ALP, PTH and urinary calcium excretion was normalized (table 8).

Case 3

A 26-year-old female, diagnosed as having CVID in 2005, was hospitalized because of fever, purulent sputum and long-lasting pain in her bones and back in January 2007. She reported recurrent upper and lower airway tract infections since childhood. The diagnosis of CVID was made on the basis of low immunoglobulin levels (IgG 139 mg/dl, IgA <24.5 mg/dl, IgM <16.5 mg/dl) and the absence of a response to vaccination (anti-*Haemophilus influenzae* antibody titer was 0.014 μ g/ml, normal: >1 , and antitetanus antibody was 0.005 IU/ml, normal: >0.1).

The medical history revealed a splenectomy in 2003 because of hypersplenism and nephrectomy because of nephrolithiasis. The patient had a history of consanguinity. She had been hospitalized 3 times/year because of respiratory tract infections since having been diagnosed with CVID.

She did not report diarrhea or weight loss on admission. Her musculoskeletal complaints were considered to be fibromyalgia. Her vital signs were as follows: temperature 37.2°C, blood pressure 120/70 mm Hg, pulse 72 beats/min, and BMI 23.5. Physical examination revealed bilateral submandibular and right axillar lymphadenopathy, crackles on auscultation in lower part of the left lung, and hepatomegaly.

Flow-cytometric analysis of the peripheral blood lymphocyte subsets and immunoglobulin levels were shown in table 3. Hb was 13.5 g/dl, leukocytes were 10,600/ μ l, neutrophil count was 2,060/ μ l, lymphocyte count was 7,000/ μ l, and monocytes 1,330/ μ l. Autoantibodies, including antitransglutaminase, anti-

Table 9. Markers related to bone metabolism of case 3

Values	On admission	Normal range
Osteocalcin, ng/ml	4.7	3.1–13.7
Deoxyipyridinoline, nM/mM	8.4	3–7.4
Urinary calcium, mg/24 h	91	100–300
25-OH vit D, ng/ml	6	10–40
1,25(OH) ₂ vit D, pg/ml	40	30–65
PTH, pg/ml	53	15–65
ALP, U/l	903	35–104

gliadin and antithyroid, were negative. Thyroid function tests were normal.

The bone marrow examination revealed small mature lymphoid cell infiltration. Blasts were lower than 5%. Immunohistochemical analysis showed that infiltrating lymphocytes were CD3+ and CD5+, but CD7– and these cells mostly consisted of CD4+ and to a lesser extent of CD8+ T cells. CD56 was reported to be negative. Lymph node biopsy revealed a remarkable lymphoid infiltration abolishing the normal architecture. Immunohistochemical evaluation showed similar results with bone marrow examination in that these cells were CD3+, CD4+, CD5+ but CD7–, CD20– and CD56–. Molecular analysis revealed that B cells were polyclonal; however, clonal patterns of T cells were found by TCR gene rearrangement. Peripheral blood flow-cytometric analysis was found as follows: CD3+ 85%, CD4+ 48%, CD8+ 35%, CD5+ 85%, CD7+ 80%, CD16+CD56+ 2%, CD19+ 12%, CD20 15%.

Significantly high ALP levels persisting for 1 year were detected accompanied by mild transaminase and remarkable GGT elevation (table 4). Intensive lymphoid infiltration, consisting of CD3+, CD8+ and CD16+ cells, was detected in a liver biopsy. Neither granuloma formation nor cholestasis was detected. Hepatitis serology was HBsAg, anti-HBs, anti-HBc-IgG, anti-HCV and HCV-RNA negative.

HRCT of the thorax revealed nodular lesions suggesting granulomas in both lower parts of the lungs. Enlarged liver with homogenous density and mesenteric, retroperitoneal and celiac adenopathies smaller than 1 cm in diameter were detected on abdominal CT.

Lymphoid infiltration of the bone marrow, lymph node and the liver, the loss of CD7 expression and monoclonality of the T cell population suggested a peripheral T cell leukemia/lymphoma developed on the basis of CVID. During the follow-up period, autoimmune hemolytic anemia developed and was treated successfully by administering prednisolone therapy.

In the light of the patient's extremely high ALP levels and complaints of muscle and bone pain of long duration, we suspected an increased bone turnover. X-ray examination of the extremities was normal. Bone mineral density was normal. Radionuclide bone scan evaluation revealed increased activity in both mandibular areas, which was considered to be related to tooth pathology. Urinary deoxyipyridinoline was increased and urinary calcium was found to be decreased. 25-OH vit D level was found to be lower than the normal range (table 9). VDR mRNA expression in PBMC and HF was found to be reduced

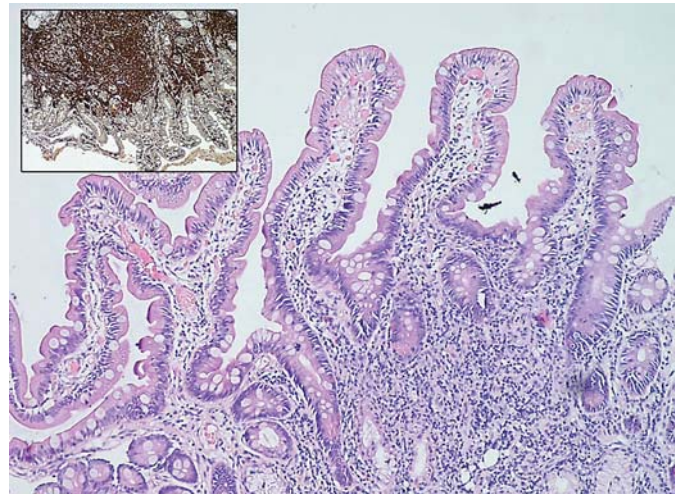


Fig. 4. Case 3. Normal-appearing duodenal villous architecture. Intraepithelial lymphocytes were within the normal limits. HE. $\times 10$. **Inset** CD3 immunostaining. Note the mucosal lymphoid aggregate predominantly composed of T lymphocytes but no increase in intraepithelial T lymphocytes was observed. CD3. $\times 4$.

(table 6). VDR gene polymorphism evaluation was performed (table 7).

Upper endoscopic examination was carried out and evaluation of the biopsy specimens taken from the antrum and the second part of the duodenum revealed diffuse lymphoid infiltration and follicles with germinal centers in lamina propria. Immunohistochemical evaluation showed mixed lymphoid infiltration consisting of CD3+ T cells and CD20+ B cells. Villous atrophy and giardiasis were not reported (fig. 4). The amount of fecal fat excretion was in the normal range (6 g/24 h).

In view of these results, calcitriol (Rocaltrol 0.25 μ g, b.i.d.) together with calcium 1 g/day was commenced.

Discussion

Osteomalacia is characterized by defective mineralization of osteoid and vit D deficiency is one of the causes of osteomalacia in the adult population [5]. It can be asymptomatic, but can also present with aching bones, muscle weakness, pelvic deformities and a waddling gait [6, 7]. Our patients presented remarkable vit D deficiency in the absence of musculoskeletal symptoms except for case 3.

The most useful findings alerting physicians to search for a preosteomalacic state are increased serum ALP and PTH levels, reduced urinary calcium excretion and low serum 25-OH vit D levels [5].

The only reliable way to determine the vit D status of an individual is to evaluate circulating concentrations of 25-OH vit D. 1,25(OH)₂ vit D measurement can never be used as an indicator of the vit D status [8, 9]. The reasons are a short half-life, very low blood levels, and increased renal 1,25(OH)₂ vit D synthesis induced by PTH leading to misinterpretation.

People living north of latitude 37°, dark-skinned people, vegetarians and veiled women run a greater risk of developing vit D deficiency [8, 10–12]. None of these risk factors were present in our patients. None of them had a history of anticonvulsant or antituberculous drug use that might be linked to a vit D deficiency [13]. The absence of lytic lesions or fractures on X-ray examination of the cranium and extremities, normal bone radionuclide scan evaluation, absence of hypercalcemia, normal or slightly increased deoxypyridinoline levels suggested that malignant osteoclast activity was unlikely.

None of the patients complained of malabsorption-related symptoms nor did they show biochemical abnormality suggestive of malabsorption. They did not have a history of gastric or intestinal operations. None of them had been given a diagnosis of gastrointestinal or pancreaticobiliary disease implicated in the development of osteomalacia such as inflammatory bowel disease or pancreatitis [13].

The absence of fever, arthritis, arthralgia, lymphadenopathy and hypocalcemia made Whipple's disease unlikely. Additionally, biopsy evaluation did not reveal foamy histiocytes in lamina propria suggestive of Whipple's disease [14]. Celiac serology was negative and biopsy appearances did not suggest celiac disease in our patients.

There have been several reports indicating malabsorption, steatorrhea, hypocarotenemia, low serum folate, and vit A and B₁₂ levels in patients with giardiasis [15, 16]. However, malabsorption resulting from giardiasis and/or lymphoid infiltration was considered unlikely as serum vit A, vit E, folate and B₁₂ levels were all found to be within the normal range in our patients.

VDR mRNA expression in PBMC and HF was found to be lower in all patients compared to the control group. vit D exerts most of its biological effects via VDRs localized in the nucleus of the cells of many tissues such as intestine, islet cells, brain, prostate and breast [8]. Additionally, immature thymocytes, and T and B lymphocytes have VDRs [8, 12] and vit D modulates T and B lymphocyte functions [17, 18]. VDRs are constitutively expressed on antigen-presenting cells [19] emphasizing the important role of vit D in the immune system.

VDR mutation can cause vit D resistance and these patients are poorly or not responsive to high-dose 1,25(OH)₂ vit D. Although these patients present all symptoms of rickets, they have very high levels of endogenous 1,25(OH)₂ vit D, normal 25 OH D vit and also show hypocalcemia and hypophosphatemia which were all not present in our patients [20].

We could not investigate the intestinal VDR expression as they refused consent for further investigations. It has been shown in experimental animal models that low intestinal VDR levels lead to vit D resistance and diminished intestinal responsiveness to 1,25(OH)₂ vit D [21]. Absence of VDR abolishes two thirds of the intestinal active calcium absorption [20]. On the other hand, a high-calcium diet has corrected the impaired bone and calcium metabolism in VDR KO mice [20]. Calcium replacement might help normalize ALP and calcium levels despite subnormal vit D levels in the first case. However, the genes regulating calcium entry into the enterocytes via epithelial calcium channels were dependent on 1,25(OH)₂ vit D [20]. Therefore, we would not have achieved an increase in calcium levels without concomitant administration of active vit D.

Exposure of 3T6 mouse fibroblasts to 1,25(OH)₂ vit D has been shown to lead to an increase of VDR mRNA concentrations and cellular VDR content [22]. Additionally, vit D ingestion has induced an increase in VDR mRNA expression in leukocytes of healthy individuals, which was proportionate to the level of circulating vit D concentration [22]. Taken together, high-dose active vit D therapy might lead to an increase in VDR and epithelial calcium channel expression of the intestinal cells, which, as a consequence, might improve the calcium absorption and contribute to the normalization of the laboratory parameters.

VDR gene polymorphism affecting VDR expression has been linked to decreased bone density, autoimmune diseases, and susceptibility to infections [23]. We examined the VDR gene polymorphism (*ApaI*, *TaqI*, *BsmI* and *FokI*) in these patients (table 7).

Studies examining the effect of VDR gene haplotypes on VDR functions have demonstrated that *BsmI*, *ApaI* and *TaqI* polymorphisms do not seem to alter VDR gene expression and VDR function [24]. *FokI* polymorphism has been associated with VDR expression and function [24] and the F allele was found to be more active in terms of its transactivation capacity [25]. On the other hand, another study evaluating the effects of immunoregulatory genes on clinical phenotype of CVID revealed that the *ApaI* A allele has been associated with higher VDR expression

than the *ApaI* a allele [26]. The patients with CVID carrying the A allele would be expected to be more sensitive to the immunosuppressive actions of active vit D. Consistent with these data, the second case who showed the lowest VDR mRNA expression had the ff and aa genotype.

1,25(OH)₂ vit D has been shown to play an important role in the innate immune system by inducing the expression of TLR coreceptor CD14, and defensin-β₂ [27]. It has also been demonstrated that TLR1/TLR2-induced cathelicidin mRNA expression is dependent on adequate 25 vit D concentration in the serum [28]. TLR activation results in the expression of VDR and 1α-vit D hydroxylase on the monocytes [2]. vit D has an important role against infections caused by organisms resistant to routine antimicrobial agents and sepsis [29].

On the other hand, vit D₃ is also the compound that downregulates TLR expression and function [2]. It has been shown that 1,25(OH)₂ vit D suppresses TNF-α production in human monocytes in a dose-dependent manner [30]. Taken together, there seems to be an interplay between TLRs and VDRs that modulates TLR-related immune responses. It seems that VDRs are necessary for a normal functioning innate immune system which is essential for a normally operating acquired immune system. Additionally, VDRs would play a role in controlling the inflammation and granuloma formation, which are two well-known complications of CVID.

vit D deficiency has been implicated in the development of some autoimmune diseases such as multiple sclerosis, type 1 diabetes mellitus, rheumatoid arthritis and Crohn's disease [23]. 1,25(OH)₂ vit D could prevent the development of type 1 diabetes by suppression of dendritic cells and Th1 cells, suppression of the antigen-presenting capacity of macrophages and dendritic cells, and promotion of Th2 lymphocytes [31].

vit D is necessary for the maintenance of glucose tolerance and insulin release; the impaired insulin secretion can be restored by means of 1,25(OH)₂ vit D replacement [32]. vit D insufficiency might have accelerated or led to the emergence of type 1 diabetes in the first case. Furthermore, as indicated in the literature, vit D deficiency might have a role in the development of polyneuropathy and sensorineural hearing loss in this patient [33]. The short period between the diagnosis of diabetes and development of neuropathy, the absence of retinopathy and the tight glycemic control which is confirmed by desirable HbA1c levels (table 4) were regarded as making diabetic neuropathy unlikely.

vit D deficiency has also been implicated in the etiology of some malignancies such as colon, prostate and

breast [8]. Untreated vit D deficiency might have a role as an additive risk factor in terms of a tendency to malignancy in patients with CVID.

We preferred giving vit D via the oral rather than the parenteral route in cases 1 and 3 in order to evaluate intestinal vit D absorption capacity. An increase in vit D and calcium levels via oral vit D replacement makes a lack of vit D absorption in the first case unlikely. Case 2 refused parenteral therapy and continued oral calcitriol replacement despite the fact that her fecal fat excretion was increased. However, interestingly, oral active vit D therapy resulted in an improvement of her laboratory parameters. This might be explained by the effect of the antibiotic treatment applied for giardiasis. We were able to maintain the serum calcium levels between 9.0 and 9.5 mg/dl with therapy in these patients. The third case could not be evaluated due to interruption of the therapy.

It is not possible to definitely say that vit D deficiency can be linked to the mechanisms responsible for emergence of immunodeficiency in these patients. Nonetheless, whatever the reason is, it has to be taken into consideration that patients with CVID may present osteomalacia in the absence of enteropathy. They may be asymptomatic and persistently high ALP levels may be the only remarkable sign. vit D deficiency may lead not only to irreparable bone loss, but also abnormalities in the innate immune system if left untreated. Additionally, it may increase the risk of inflammation, autoimmunity and malignancy in patients with CVID.

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