



Research Article

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## Evaluation of Growth and Bone Metabolism in Adolescent Patients with Common Variable Immunodeficiency

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### Abstract

**Background:** Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency, characterized by hypogammaglobulinemia, recurrent infections, chronic inflammation, nutritional deficits that may lead to osteoporosis.

**Aim:** To evaluate bone mineral density (BMD) of patients with CVID using dual energy X-ray absorptiometry (DEXA).

**Methods:** Bone mineral status of 25 CVID patients with a mean age of  $170.3 \pm 65.8$  months was examined. Bone mineral density (BMD) of lumbar vertebrae was determined by dual energy X-ray absorptiometry (DEXA) and Z scores according to age and height were compared to sex and ethnic specific reference data. Risk factors associated with decreased bone density were evaluated. Patients were examined totally as a whole group and they were also divided into two subgroups as severe and moderate CVID.

**Results:** In the total study group, mean values of weight, height, body mass index (BMI) SDS and L1-L4 Z score were  $-0.68 \pm 1.22$ ,  $-0.97 \pm 1.6$ ,  $-0.25 \pm 1.3$  and  $-1.06 \pm 1.3$ , respectively. L1-L4 Z score was decreased in the severe CVID group  $-1, 72 \pm 0, 98$  versus  $0, 45 \pm 1, 34$ .

**Conclusion:** Altered bone mineral density is an emerging health problem in CVID patients especially in the severely affected subgroup. Bone density should be evaluated according to height or puberty of the patients when delayed puberty or short stature is observed in CVID patients.

### Introduction

Common variable immunodeficiency (CVID) is the most common symptomatic primary immunodeficiency, resulting in increased susceptibility to infections and diminished responses to protein and polysaccharide vaccines [1,2]. Most common infections are sinopulmonary caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and sometimes mycoplasma [3-5]. An intrinsic B cell defect; inability to differentiate to plasma cells and secrete immunoglobulins, has been most commonly observed in terms of pathogenesis. Many defects in T cells have also been noted in patients with CVID [6].

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Osteoporosis is a systemic pathology of the skeleton characterized by loss of bone mass, decreased bone mineral density and loss of microarchitectural integrity. It is accepted as a heterogeneous condition which can occur in any age of life and its etiology attributed to multiple factors [7].

Bone metabolism is regulated by interplay between various hormones, growth factors, and cytokines. Proinflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF- $\alpha$ ) and IL-6, have roles in the regulation of immune responses, but are also known to be potent inducers of osteoclastic bone resorption *in vitro*, and may cause bone loss *in vivo*. These cytokines may also inhibit bone formation *in vitro* through their negative effects on osteoblasts, may enhance the production of other proinflammatory mediators from these cells, leading to further promotion of bone resorption by enhancing osteoclast formation and activity [8].

Recently, increased prevalence of osteoporosis has been reported in CVID patients [9,10]. Various systemic disorders observed in CVID patients such as chronic malnutrition, celiac-like disease, chronic liver and pulmonary diseases may be associated with osteoporosis. In addition, some of the patients with CVID were shown to have markedly elevated serum levels of TNF- $\alpha$  and IL-6, related with enhanced bone resorption [10,11]. These data show that altered immunological factors also play an important role in impaired bone metabolism.

In this study, we aimed to evaluate bone mineral density (BMD) of CVID patients using dual energy X-ray absorptiometry (DEXA). In addition, growth parameters and puberty were also assessed. Correlations between demographical data and immunological findings of the patients with anthropometric measures and bone mineralization were analyzed.

### Materials and Methods

Twenty-five patients admitted to Ege University Pediatric Immunology Department were analyzed retrospectively. The ethical committee approved the study and all participants gave written informed consent. All patients fulfilled criteria for CVID based on the European Society for Immunodeficiencies (ESID) definition. These criteria include a marked decrease of IgG (of at least 2 standard deviations (SDs) below the mean for age) and reduced serum IgA and/or IgM, specific antibody deficiency, age greater than two years, and exclusion of other causes of hypogammaglobulinemia [12]. Patients less than 2 years of age were excluded because of a possible diagnosis of transient hypogammaglobulinemia of infancy. Patients were examined totally as a whole group; they were also divided into two subgroups due to previously published disease severity criteria for CVID [13,14].

Severe disease group (SDG) inclusion criteria: patients who have splenomegaly and/or granulomatous disease and/or bronchiectasis and/or lower baseline IgG values (lower than 270 mg/dl on admission). This group of patients often have poor outcome and need to be hospitalized for the treatment of their pulmonary and gastrointestinal infections (n:11). Moderate disease group (MDG) CVID patients were diagnosed as fulfilling ESID criteria but not the severe disease

group inclusion criteria. Their infections were cured mostly at out-patient follow-up clinics (n:14).

Clinical information was obtained for each CVID subject from their medical records. In addition to clinical evaluation, they were examined for serum immunoglobulin levels and lymphocyte phenotyping. Serum IgG, IgA and IgM were analyzed quantitatively by Dade Behring BNII Nephelometer, Siemens, Germany and were investigated in comparison to Turkish age-related normal levels [15]. Lymphocyte subpopulations were analyzed with four-color flow cytometry using whole blood and monoclonal antibodies against CD3, CD4, CD8, HLA-DR, CD19, CD16 and CD56. All monoclonal antibodies were derived from Becton Dickinson and the polyclonal goat F(ab')<sub>2</sub> anti-human immunoglobulin antibodies from Southern Bio technology Associates, Birmingham, AL. Cells were analyzed on a FACSCalibur using CellQuestPro data analysis software (Becton Dickinson) after erythrocyte lysis. The following lymphocyte subpopulations were determined: total T lymphocytes (CD3+), total B lymphocytes (CD19+), T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), natural killer (NK) cells (CD3-16+56+) and active T cells (CD3+HLA-DR+).

Height was measured without shoes with a wall mounted Harpendenstadiometer. Weight was measured without shoes on a standard balance nearest to 100 g on a digital scale. Height and weight SDS of patients were determined according to Turkish children's standards [16]. Pubertal development was evaluated according to the method of Tanner [17] and bone age [18]. Delayed puberty is defined as no breast development by 13 years of age in a girl and no testicular enlargement by 14 years in a boy. BMD of the lumbar spine and femoral neck was measured with dual energy X-ray absorptiometry (DEXA, Hologic QDR 4500A, Waltham, MA, USA) (g/cm<sup>2</sup>). The child was supine and physiological lumbar lordosis was flattened by elevation of the knees during measurement of the lumbar spine. The results of BMD were compared to healthy age- and sex-matched Turkish children and Z-scores were calculated [19]. Height matched BMD Z scores were used to reduce the influence of bone size in patients with a height SDS below -2 SDS. Patients with BMD Z-score less than -2 were diagnosed as having low bone mass, while others were considered normal.

## Statistical Analysis

All data are expressed as mean plus or minus SD except when indicated otherwise. SPSS 15.0 program was used for statistical analyses. Correlation comparisons between paired samples were made by Pearson's product moment correlation coefficient. Statistical comparisons of numeric data were made using chi-square (Fisher's exact test) and t tests. Differences between groups were considered significant at p<0.05.

## Results

Mean age was 170.3 ± 65.8 months and there were 23 (92%) males and 2 (8%) females in the study group. Age at onset of symptoms and age at diagnosis were compared between SDG and MDG (Table 1) and results of the whole CVID group were also listed (Table 1). The patients began to have recurrent infections after 4 years of age (50 ± 45.7 months) and diagnostic delay in this cohort was about two years. The mean follow-up period was 84.4 ± 53.6 months. Eight CVID case-patients (six of them in SDG, two of them in MDG) had a positive family history of immunodeficiency. Seven of the 25 CVID case-patients had consanguineous parents (28%) and this ratio was as higher, 54.5 % in SDG (n:6) and 7.1% in MDG (n:1) according to disease severity groups.

The baseline serum IgG was 373.3 ± 196.7 mg/dl at the time of diagnosis in whole CVID group before the initiation of IVIG therapy. Serum IgG and IgM levels did not show any significant difference between severe and moderate groups. However, serum IgA was significantly low (p<0.05) in SDG compared to MDG (Table 1). Three immunoglobulin levels (IgG, IgM, IgA) together were determined to be lower in 14 (56%) patients than age-matched control levels. White blood cell (WBC) counts, absolute lymphocyte counts (ALC), CD3+ T cells, CD19+ B cells, CD3+CD4+ T helper cells, CD3+CD8+ T cytotoxic cells, CD3-CD16+CD56+ natural killer cells and HLA-DR+ active T cells numbers did not show any significant difference between severe and moderate groups of CVID patients (Table 1). Major lymphocyte subset percentages and absolute counts were in normal levels compared to healthy Turkish children lymphocyte subsets [20].

**Table 1:** Patients' characteristics, serum immunoglobulins and lymphocyte subsets in whole CVID group and in two subgroups according to disease severity.

	Severe disease Group (SDG) n:11	Moderate disease Group (MDG) (n:14)	P (SDG vs MDG)	All patients (n:25)
Age (months)	184 68.1	159.4 ± 64.3	>0.05	170.3 ± 65.8
Age at onset of symptoms (months)	54 ± 49.3	46.6 ± 44	>0.05	50 ± 45.7
Age at diagnosis (months)	76.3 ± 55.6	80.3 ± 55.7	>0.05	78.5 ± 54.5
Family history (n)	6	2	<0.05	8
Consanguinity (n)	6	1	<0.05	7
IgG (mg/dl)	333 186.5	476.7 ± 242.7	0.119	373.3 ± 196.7
IgM (mg/dl)	40.2 29.1	56.4 ± 32.2	0.609	44.5 ± 26.4
IgA (mg/dl)	5.4 0.9	49.9 ± 48.7	<0.05	30.2 ± 22.3
WBC/ mm <sup>3</sup>	11680 ± 7931	8629 ± 4002	0.222	9971 ± 5012
ALC/mm <sup>3</sup>	4228.6 ± 3783.7	2876.3 ± 1956.8	0.273	3572 ± 2456
CD3+ T cells/mm <sup>3</sup>	2759.3 ± 2566.7	1970.9 ± 1265.9	0.343	2477 ± 1996
CD3+CD4+/mm <sup>3</sup>	1042.6 ± 782.5	1070.7 ± 755.2	0.931	1062 ± 744
CD3+CD8+ / mm <sup>3</sup>	1690.4 ± 1917	896.1 ± 784	0.188	1284 ± 987
CD16+56+ /mm <sup>3</sup>	554.6 ± 826.8	437.4 ± 419.3	0.675	476 ± 524
HLA DR+ T /mm <sup>3</sup>	546.7 ± 703.7	271.3 ± 239	0.219	373 ± 404
CD19+ B cells/mm <sup>3</sup>	7.2 ± 4.2	9.2 ± 4.9	0.297	8.3 ± 4.6
Adequate specific antibody response (n)	4	10	0.386	14

Specific antibody response was found to be positive in 56% (n=14), 36% (n=4), and 71% (n=10) of the whole, severe disease and moderate disease groups, respectively.

Mean values of weight, height and body mass index (BMI) SDS were  $-0.68 \pm 1.22$ ,  $-0.97 \pm 1.6$  and  $-0.25 \pm 1.3$ , respectively, in the whole group. L1-L4 Z score was  $-1.06 \pm 1.3$ . Weight, Height, Body Mass Index (BMI) SDS and L1-L4 Z score were also compared between SDG and MDG. Weight SDS (p=0.003), height SDS (p=0.001) and L1-L4 Z scores (p=0.016) were determined to be significantly decreased in SDG. BMI SDS was not different between two groups (p=0.489) (Table 2). The percentage of patients with BMD Z score less than -2 was found to be 29.2% (n=7), 45.5% (n=5) and 15.4% (n=2) in total, severe disease and moderate disease groups respectively.

Pubertal status of CVID case-patients were evaluated. Ten patients (40%) within the study group were in prepubertal period. Six patients (24%) showed normal stage of puberty for age, whereas 9 (36%) had delayed puberty. Pubertal stages were compared between severe and moderate group after the exclusion of prepubertal patients. In SDG, six patients had delayed, one had normal puberty. In MDG, 3 patients had delayed, 5 had normal pubertal stage for age (p=0.057).

Serum IgA level showed statistically significant correlation between weight SDS and height SDS (p=0.028, R=0.469, p=0.044, R=0.434 respectively) in the whole group, CD3+CD8+ T cytotoxic cell ratio showed negative correlation between weight SDS and BMI SDS (p=0.024, R=-0.502 and p=0.006, R=-0.589 respectively). In SDG, L1-L4 Z score negatively correlated with CD3+CD4+ T cell ratio (p=0.037, R=-0.738). Patients with positive family history had lower L1-L4 Z score (p=0.029) and those born from consanguineous parents had lower weight SDS, height SDS and serum IgA level (p=0.011, p=0.026 and p=0.039, respectively) as these patients were mostly in SDG.

## Discussion

We hypothesized that various pathological events experienced by CVID patients over many years in childhood might lead to defects in bone mineralization and growth. In this cohort of 25 CVID patients, growth retardation, osteoporosis and delayed puberty are clinical and laboratory disorders often observed in the severe group of patients with life-threatening complications. These endocrinologic problems are not only associated with disease duration, but also with some immunological factors.

**Table 2:** The evaluation and comparison of two groups of patients for osteoporosis and growth parameters.

	Severe Disease Group n (%)	Moderate Disease Group n (%)	P	Total CVID Patients n (%)
	11	14		25
Delayed puberty n (%)	6 (54.5)	3 (21.4)	0.057	9 (36)
Normal puberty n (%)	1 (9.1)	5 (35.7)		6 (24)
Prepubertal n (%)	4 (36.4)	6 (42.9)	0.527	10 (40)
Short stature n (%)	6 (54.5)	0 (0)	0.001	6
L1-L4 BMD Z score	$-1.72 \pm 0.98$	$-0.45 \pm 1.34$	0.016	$-1.06 \pm 1.3$
Weight SDS	$-1.42 \pm 1.1$	$-0.02 \pm 0.94$	0.003	$-0.68 \pm 1.22$
Height SDS	$-2.11 \pm 1.41$	$-0.10 \pm 1.17$	0.001	$-0.97 \pm 1.6$
BMI SDS	$-0.40 \pm 1.45$	$0 \pm 0.37$	0.489	$-0.25 \pm 1.3$

Rivoisy et al. have reported that parental consanguinity is associated with severe phenotype and increased complications in CVID [13]. Splenomegaly, granulomatous disease, bronchiectasis, opportunistic infections were observed in a high incidence in CVID patients with parental consanguinity compared to others without parental consanguinity. Similar to Rivoisy et al. [13] study, we found parental consanguinity as 54.5% in SDG who had splenomegaly and/or granulomatous diseases and/or bronchiectasis and/or very low IgG values. In our study, patients with positive family history had lower L1-L4 Z score and patients born from consanguineous parents had lower weight SDS, height SDS and serum IgA level. These data suggested that consanguineous CVID patients are mostly clinically severe cases with growth retardation and osteoporosis.

Delayed diagnosis and treatment can lead to bronchiectasis and severe complications in CVID patients [21]. In our study, diagnostic delay was approximately 2 years. Although diagnostic delay was not so long, 44% of patients were already in SDG presenting with complications such as bronchiectasis (44%) and splenomegaly (36%) on admission, and delayed puberty (54.5%) during follow-up.

A very low IgA (<10 mg/dl) may be another parameter to predict the severity of CVID patients, because serum IgA in SDG CVID patients ( $5.4 + 0.9$  mg/dl) was significantly lower than the ones in MDG ( $49.9 + 48.7$  mg/dl) according to our data. Serum IgA level showed statistically significant correlation between weight SDS and height SDS. This correlation clarifies the relationship between recurrent infections, growth retardation and bone metabolism.

Several mechanisms such as recurrent infections, chronic lung diseases, chronic inflammation, chronic nutritional defects and associated gastrointestinal abnormalities, immunological alterations, problems observed in chronic illnesses, chemotherapeutic and immunomodulatory drugs including steroids were suggested to explain the susceptibility of these patients for defective bone mineralization, short stature and delayed puberty.

Bone continues to play a role in adaptive immunity, beyond its influence on lymphocyte development [22]. T and B lymphocytes are central components of the immune system that facilitate recognition and destruction of pathogens. Long-lived memory T and B cells return to specialized niches in the bone marrow [22]. Mice lacking either B cells or T cells have osteoporotic bones suggesting that these immune cells participate in the maintenance of bone homeostasis [23]. In addition, mature B cells produce more than 50% of total bone marrow derived osteoprotegerin (OPG) which would contribute to restraining osteoclastogenesis during normal physiology [24]. The physiologic importance of OPG has been shown by the fact that overexpression of OPG in mice results in severe osteopetrosis while OPG null mice are osteoporotic [24]. OPG levels were found to be elevated in CVID patients significantly correlated with increased TNF- $\alpha$  levels [8]. Enhanced OPG levels may be a compensatory response to enhanced osteoclast activity and may possibly be correlated to enhanced activity of other members of TNF family [8].

Furthermore, Ueland et al. showed that CVID patients had significantly higher serum levels of CTX-I (carboxyterminal crosslinking telopeptide of type I collagen) and B-ALP (bone-specific alkaline phosphatase) and significantly lower serum levels of IGF-I and IGFBP-3 compared to controls [10]. Persistent immune activation *in vivo*, with raised levels of proinflammatory cytokines, may be related to disturbed bone homeostasis in CVID patients,

further supporting an interaction between immune related mediators and bone metabolism in humans.

Chronic illness is frequently accompanied by osteopenia which is caused by a combination of factors related both to the primary illness itself as well as to secondary disturbances in the endocrine system [25]. For example, in a longitudinal study, it has been shown that the greatest decrease in BMD was observed during the first 6 months following renal transplantation [26]. In addition, many drugs alter bone remodeling and may lead to bone loss.

In conclusion, this study demonstrated that severe group of CVID patients develop altered bone mineralization, growth retardation and delayed puberty. These disorders have to be managed carefully in order to increase the quality of life. Further studies are needed to identify the exact mechanisms leading to decreased bone density in CVID patients.

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