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Low serum levels of 25-hydroxy vitamin D in adults affected by thalassemia major or intermedia☆

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Abstract

Adult thalassemic patients have reduced bone mass due to disturbances in several different mechanisms affecting bone turnover. To determine if vitamin D deficiency contributes to the low bone mass of adult thalassemic subjects, we studied serum 25-OH-vitamin D levels in 90 patients (age ranging between 21 and 48 years) affected with thalassemia major (TM) and 35 (age 21–56 years) with thalassemia intermedia (TI).

TM patients had been receiving regular transfusions from the age of 2 years and had increased serum ferritin, glutamic oxalacetic transaminase, glutamic piruvic transaminase as well as low bone density (L1–L4 *Z* score −2.07 ± 0.2). TI patients did not receive transfusions, but their ferritin levels were increased as well (520.3 ± 138,1). 8 TM patients (10.1%) and 4 TI (11.4%) had serum 25-OH-vitamin D less than 10.4 ng/ml and were considered presenting an absolute deficiency of vitamin D. Mean 25-OH-vitamin D was significantly ($P < 0.01$) lower in both TM and TI patients (20.3 ± 0.7 ng/ml and 20.9 ± 2.3 ng/ml, respectively) than in 100 healthy control subjects of similar age (25.2 ± 1 ng/ml). 1,25-OH-vitamin D levels were in the normal–lower levels (45.15 ± 1.5 mg/dl), while 24 H urinary calcium was below the normal range (15.75 mg/dl). In TM patients, the 25-OH-vitamin D levels correlated negatively with age ($P < 0.05$) and with serum ferritin ($P < 0.05$). TM and TI patients with low 25-OH-vitamin D levels (<17.8 ng/ml) presented higher serum ferritin levels ($P < 0.01$) and higher PTH ($P < 0.05$) compared to those with normal vitamin D. Moreover, TM patients with low 25-OH-vitamin D levels were significantly older ($P < 0.05$) and had higher GPT ($P < 0.05$) than patients with normal vitamin D.

In conclusion, calcium metabolism is frequently impaired in adult thalassemic patients. An early and effective medical treatment should be taken in consideration by the clinician in order to improve the bone health in these patients.

Keywords: Vitamin D; Osteoporosis; Osteopenia; Thalassemia major; Iron overload

Introduction

Many studies have shown low bone mass in adult patients with thalassemia [1–4]. In a large study, adult thalassemia major patients (TM) presented 51% prevalence of osteoporosis and 45% prevalence of osteopenia [2].

Because many adult TM patients have a hypogonadotropic hypogonadism [5], it has been suggested that their reduced bone mass is primarily depending from sex hormone deficiency [6,7]. Although confirmed by our own data [8], we have also observed that, at least in women with thalassemia major, hormone replacement therapy is unable to prevent bone loss [8]. It adds evidence to the opinion that several mechanisms potentially contribute to their low bone mass [9].

One of these mechanisms may be vitamin D deficiency. Notably, TM patients progressively develop iron overload, and it is possible that a deficiency in liver hydroxylation of vitamin D, or in vitamin D absorption, appears in older thalassemic patients. However, studies in children [10,11] and in adult thalassemic patients [3,11–15] have shown different results. Recently, Voskaridou et al. [3], evaluating 45 adult TM patients reported serum vitamin D (25-OH and 1,25-OH-vitamin D) levels were within normal limits in almost all patients. Conversely, Pratico et al. [12] observed that 32 of 113 thalassemic patients (including children and adults) had low serum levels of 25-OH-vitamin D.

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While it is difficult to explain these differences, no determination of appropriate controls of similar age was reported.

To re-evaluate the possibility that a deficiency of vitamin D hydroxylation contributes to reduced bone mass in adult thalassemic subjects, we measured serum 25-OH-vitamin D in 90 TM patients with age ranging between 21 and 41 years old and 35 patients affected with thalassemia intermedia. We also explored any correlations between circulating 25-OH-vitamin D and iron overload and/or liver damage. For comparisons, we calculated the normal ranges of serum 25-OH-vitamin D in our healthy control population of similar age. Because serum values of 25-OH-vitamin D are influenced by sun exposition and may change depending on the season [25], all patients and controls were studied during the same period of the year (January–April).

Materials and methods

Subjects

90 TM patients (53 women and 37 men) and 35 TI patients were studied. They had a mean age of 26.7 ± 0.6 years old (range 21–41 years). Mean BMI was 23 ± 0.5. All TM patients had been receiving regular transfusions after 2 years of life.

57 patients (41 women and 16 men) were on hormonal replacement therapy due to hypogonadotropic hypogonadism. 13 TM patients were also on treatment with thyroxine for hypothyroidism.

100 normal subjects (70 women and 30 men, among young doctors and nurses) of similar age and weight (mean age 26.7 ± 0.2 years old, mean BMI 23 ± 1) were used as controls.

In patients and controls, after an overnight fasting, blood samples were obtained. Serum levels of 25-OH-vitamin D, parathyroid hormone (PTH), ferritin, glutamic oxalacetic transaminase (GOT), and glutamic piruvic transaminase (GPT) were measured. All samples were taken during the January–April. In all patients and controls, informed consent was obtained, and the study was approved by the local ethical committee.

Palermo latitude is 38Â° 09′ North and is on the Mediterranean Sea. All our patients and controls had at least 3 h a day of sun exposition. Calcium intake was 1328.78 ± 21.2 mg/day for TM patients, 1130.21 ± 11.4 mg/day for TI patients and 1252.36 ± 12.5 for the controls. None of the patients or controls was treated with vitamin D or calcium supplements.

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In TM patients, bone mineral density of the lumbar spine (L2–L4) was determined using dual X-ray absorptiometry (DEXA, Lunar DPX-Plus).

Biochemical analysis

25-OH-vitamin D (normal range 30–60 ng/ml) was evaluated by enzyme-linked immunosorbent assay (ELISA), while 1.25-OH-vitamin D by radioimmunoassay (normal range 48–110 pg/ml) using materials provided by Immunodiagnostic Systems (Boldon, United Kingdom). Intact PTH (normal range 15–60 ng/ml) was measured using an enzyme-linked immunosorbent assay (ELISA) with materials provided by Biosource, Belgium. Ferritin was determined by ELISA using materials provided by RAMCO (TX, USA). GOT and GPT were measured by spectrophotometric method.

In all assays, the intra-assay coefficient of variation was 6% or less, and the interassay coefficient of variation was 15% or less.

Analysis of the 24-h urine collections for the magnesium and calcium levels (normal ranges: 75–125 mg/dl; 100–300 mg/dl, respectively) were performed using Locorotondo Labs kits (Palermo, Italy).

Statistical analysis

Analysis of variance and the Mann–Whitney *U* test was used to compare patients and controls. Correlations were calculated using Pearson correlation index. *P* less than 0.05 was considered statistically significant.

An absolute deficiency of vitamin D was diagnosed in patients with blood levels of 25-OH-vitamin D lower than mean −2 SD of normal controls. Patients presenting blood 25-OHvitamin D values between mean −1 SD and mean −2 SD were considered affected by partial vitamin D deficiency. Results are expressed as mean ± SE.

25-OH-vitamin D levels were also analyzed in according with Chapuy, Malabanan, and Heaney findings and considered normal if above 30 ng/ml, reduced if below [17–19].

Results

Normal controls had serum 25-OH-vitamin D levels of 25.2 ± 1 ng/ml. SD was 7.4 ng/ml and serum levels <10.4 ng/ml (mean −2 SD) were considered indicative of 25-OH-vitamin D deficiency, whereas serum levels <17.8 ng/ml suggested a relative deficiency of 25-OH-vitamin D.

Mean serum level of 25-OH-vitamin D was significantly ($P < 0.01$) lower in TM and TI patients than in controls (Table 1).

8 TM patients (10.1%) had serum 25-OH-vitamin D less than 10.4 ng/ml and were considered presenting an absolute deficiency of 25-OH-vitamin D. 28 TM patients (30.4%) had serum 25-OH-vitamin D less than 17.8 ng/ml but higher than 10.4 ng/ml and were considered having a subclinical deficiency of 25-OH-vitamin D. In total, 45.57% of adult TM patients had a vitamin D deficiency. 13 TI patients (37.1%) had serum 25-OH-vitamin D between 10.4 ng/ml and 17.8 ng/ml, while 4 of them had values below 10.4 ng/ml.

1.25-OH-vitamin D levels were measured in TM patients and were at the normal–lower range (55.4 ± 2.1 pg/ml).

Calcium serum levels were at the lower normal range in TM patients (8.93 ± 0.06) and were reduced compared to controls (9.36 ± 0.05), although a statistical significance was barely reached. Calcium serum levels were negatively correlated with ferritin levels ($r = -0.32$, $P = 0.028$).

Table 1
Serum levels of 25-OH-vitamin D (ng/ml), PTH (ng/ml), ferritin (ng/ml), GOT (ui/ml), and GPT (ui/ml) in 90 adult TM patients, 33 TI patients and 100 normal controls of similar age

	25-OH-vitamin D	PTH	Ferritin	GOT	GPT
Controls	25.2 ± 1	36 ± 0.8	144 ± 11	12 ± 1	10 ± 1
TM patients	20.3 ± 0.7**	24 ± 1	717 ± 36**	27 ± 1**	29 ± 1**
TI patients	20.9 ± 2.3**	53.26 ± 4.3*	520.3 ± 138.1*	23.8 ± 3.35*	30.1 ± 3.78**

* $P < 0.05$ vs. controls.
** $P < 0.01$ vs. controls.