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ORIGINAL ARTICLE

Cardiovascular dysfunction and vitamin D status in childhood acute lymphoblastic leukemia survivors

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Abstract

Background Vitamin D (25-OHD) has a role in bone health after treatment for cancer. 25-OHD deficiency has been associated with risk factors for cardiovascular disease, but no data focusing on this topic in childhood cancer survivors have been published. We investigated the 25-OHD status in children treated for acute lymphoblastic leukemia (ALL), and evaluated its influence on vascular function.

Methods 25-OHD levels were evaluated in 52 ALL survivors and 40 matched healthy controls. Patients were grouped according to 25-OHD level (< 20 ng/m or ≥ 20 ng/ml). Auxological parameters, biochemical and hemostatic markers of endothelial function (AD, HMW-AD, ET-1, vWFAg, TAT, D-dimers, Fbg, and hs-CRP), ultrasound markers of vascular endothelial function (flow-mediated dilatation, FMD, common carotid intima-media thickness, C-IMT, and antero-posterior diameter of infra-renal abdominal aorta, APAO) were evaluated in the patients.

Results Cases showed higher prevalence of 25-OHD deficiency than controls ($p = 0.002$). In univariate analysis via mean comparisons, 25-OHD deficient (< 20 ng/ml) patients showed higher C-IMT values compared to the 25-OHD non-deficient (≥ 20 ng/ml) group ($P = 0.023$). Significant differences were also found for ET-1 ($P = 0.035$) and AD-HMW ($P = 0.015$). In the multiple regression models controlling for some confounders, 25-OHD still was associated with C-IMT ($P = 0.0163$), ET-1 ($P = 0.0077$), and AD-HMW ($P = 0.0008$).

Conclusions Childhood ALL survivors show higher prevalence of 25-OHD deficiency as compared to controls. The 25-OHD levels appear to be linked to indicators of endothelial and vascular dysfunction. Careful monitoring of 25-OHD balance may help to prevent cardiovascular diseases in childhood ALL survivors, characterized by high cardiovascular risk.

Keywords Acute lymphoblastic leukemia · Cardiovascular disease · C-IMT · Vascular ultrasound studies · Vitamin D

Introduction

Vitamin D is traditionally known for its role in bone metabolism, and has been widely investigated in cancer survivors [1–4]. Childhood cancer survivors have a higher risk of vitamin D deficiency mainly due to their increased sedentary activities, more time spent indoors, poor diet and exposure to chemotherapy. Moreover, they are prone to dysbiosis due to extensive use of antibiotics during chemotherapy. Vitamin D is important to maintain gut microbiota in a state of eubiosis [5], and to prevent metabolic syndrome and subsequent cardiovascular disease [6].

Recently, vitamin D deficiency has been associated with various risk factors for cardiovascular disease, including obesity, hypertension, dyslipidaemia, insulin resistance, and metabolic syndrome [7–11]. Furthermore, vitamin D exerts

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some effects on endothelial homeostasis [12], and is known to improve endothelial dysfunction [13, 14]. As previously reported, patients treated for childhood acute lymphoblastic leukemia (ALL) are prone to endothelial dysfunction, metabolic syndrome and cardiovascular disease due to therapy-related sequelae and lifestyle changes [15, 16]. In particular, cardiovascular and endothelial dysfunction can manifest itself right after the end of the ALL treatment, and not only as a late effect of the therapy, regardless of overweight or obesity [17]. No previous studies have focused on the role of vitamin D in cardiovascular risk in childhood cancer survivors. In this study, for the first time we investigated the vitamin D status of patients treated for childhood leukemia; and we evaluated the influence of vitamin D levels on the delicate balance of vascular function in these patients. This work contributes to improving knowledge about the pathogenetic mechanisms behind the development of vascular dysfunction as well as targeting strategies to prevent it.

Methods

Study population

The sample consisted of 52 childhood ALL survivors (19 males), 9.7 ± 4.1 (range 4.2–19.4) years old, recruited at the Pediatric Hematology and Oncology Clinic, University Hospital of Bari. Patients received chemotherapy according to the ongoing international AIEOP-BFM ALL protocols. According to risk group classification of ALL, 18 patients were at standard risk, 29 patients medium risk and 5 patients high risk. The average interval of time lasting from end of chemotherapy was 28.2 months (range 4–102 months). Inclusion criteria were: (a) age ranging from 3 to 20 years old; (b) diagnosis of ALL; (c) end of antineoplastic therapy since at least 3 months previously; (d) complete remission of ALL. Exclusion criteria were: (a) endocrine and/or metabolic disorders prior to ALL diagnosis; (b) cardiovascular disorders prior to ALL diagnosis; (c) genetic syndromes; (d) past use of drugs, alcohol consumption or smoking; (e) family history of cardiovascular disease in parents or grandparents (ischemic heart disease or cerebral vascular disease). Patients did not take vitamin D supplementation before or during the study period, according to the policy of our centre. The control group consisted of 40 children, matched to the patients by age, body mass index (BMI) and sex, referred to our Department of Pediatrics for minor surgery or for minor trauma to head or limbs or for chest pain. All subjects were in good general health and had not been taking drugs or vitamin D supplementation for the previous 3 months (Fig. 1).

All the procedures used were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation. The study protocol was approved by the local Institutional Review Board. All participants, or their parents when below 18 years old, gave their written informed consent before their inclusion in the study.

Auxological evaluation

Height and weight were measured, and BMI was calculated and expressed as kg/m^2. Systolic (SBP) and diastolic blood pressures (DBP) were also measured.

Biochemical assessments

Blood samples for the determination of 25-OH vitamin D (25-OHD), glycaemia, insulin, total cholesterol (TC), high- (HDL-C) and low- (LDL-C) density lipoprotein-cholesterol, triglycerides (TG) and high sensitive C-reactive

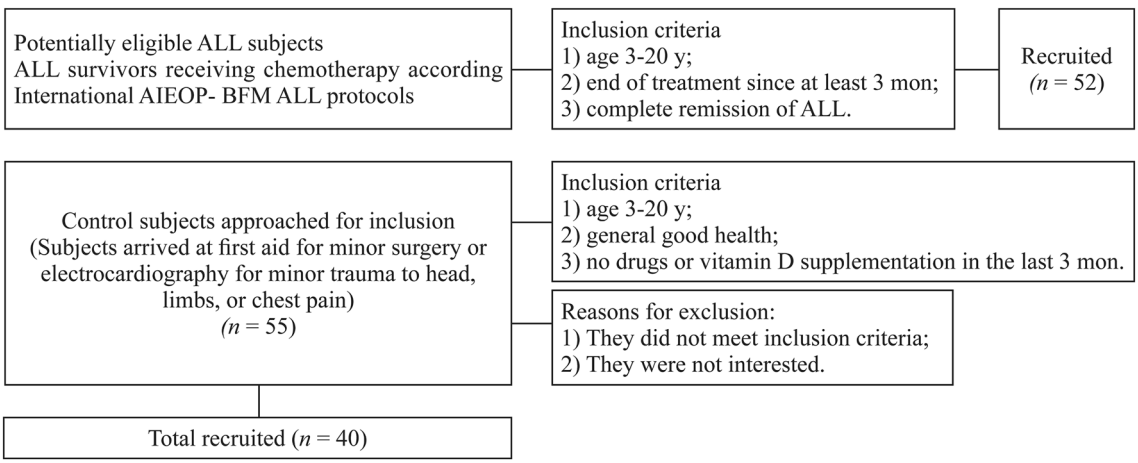

Fig. 1 Flow chart of recruitment of cases and controls

protein (hsCRP) were taken after overnight fasting in all subjects during routine blood sampling. Insulin resistance was calculated by the Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) using the formula = fasting plasma glucose (mmol/L) × fasting plasma insulin (mU/mL)/22.5. 25-OHD was measured using 25-OH Vitamin D Total ELISA, ALPCO (Salem, NH, USA) assay. 25-OHD deficiency was defined as a level < 20 ng/mL according to the recent guidelines proposed by the Institute of Medicine (IOM) [18].

Patients were grouped according to the 25-OHD level as 25-OHD deficient (25-OHD < 20 ng/mL) or non-25-OHD deficient (25-OHD ≥ 20 ng/mL).

Hemostatic markers

Endothelin-1 levels were measured by ELISA (R&D System Europe, Lille, France). The total adiponectin and multimeric high-molecular weight (HMW) subfractions were measured using a commercial ELISA (ELISA 47-ADPH-9755; ALPCO Diagnostics, Salem, VT, USA). The von Willebrand factor (vWF), as vWF antigen, and D-Dimer concentrations were measured by ELISA (Asserachrom Diagnostica, Stago, France). Thrombin–antithrombin complex (TAT) was measured with a commercial ELISA (Enzygnost TAT micro). Fibrinogen was measured in citrate plasma with a clot-rate assay using ACL 200/IL instrument (Instrumentation Laborator, Milan, Italy).

Vascular ultrasound studies

We performed vascular ultrasound measurements, including flow-mediated dilation (FMD), common carotid intima-media thickness (C-IMT), antero-posterior abdominal aorta diameter (APAO) in the Cardiology Department, as previously described [19–21].

Statistical analysis

Statistical analyses were run within the R environment [22]. Descriptive statistics were expressed as mean values ± standard deviation (SD). Auxological measurements were standardized by age and sex and reported in SD score (SDS) in keeping with Italian growth charts with GrowthCalculator3®.

Crude (unadjusted) comparisons between means were performed via *t* tests and the Fisher exact test was used for comparisons between percentages and associations between categorical variables: two-sided *P* values were reported. A *P* value < 0.05 was considered statistically significant.

Multivariable analyses controlling for possible confounders, were carried out via regression modelling to assess the vitamin D effect on the outcomes of interest. Due to the nonlinear confounding effects, additive linear regression models with penalized spline components were fitted [23]. *P* values were computed via likelihood ratio tests.

Results

The study population consisted of 52 patients, matched to 40 controls by age (9.7 ± 4.1 years vs. 10.5 ± 4.0 years, $P = 0.36$) sex (M/F 19/33 vs. 16/24, $P = 0.20$) and BMI (BMI SDS 0.9 ± 0.93 vs. 0.89 ± 0.82, $P = 0.90$).

Prevalence of 25-OHD deficiency

The prevalence of 25-OHD deficiency was significantly higher in cases compared with controls (62.2% vs. 15%, $P < 0.001$). Figure 2 shows the distribution of 25-OHD levels in cases and controls.

25-OHD deficiency and ALL risk group classification

According to ALL risk group classification, 25-OHD was < 20 ng/mL in 13/18 standard risk, in 15/29 intermediate risk and in 5/5 high risk patients. No statistically significant difference was found between the three ALL risk groups ($P = 0.08$).

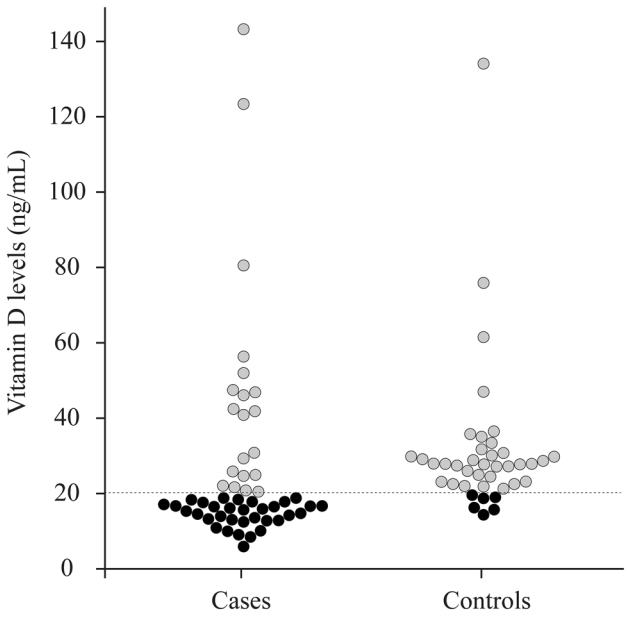

Fig. 2 Distribution of 25-OHD levels in cases ($n = 52$) and controls ($n = 40$). The full circles represent subjects with vitamin levels less the threshold 20 ng/mL (dashed line)

25-OHD deficiency and vascular function

Table 1 shows the main clinical and biochemical characteristics of patients. Corresponding mean values and standard deviations for all the observed variables are reported along with *P* values testing for mean difference.

OHD deficiency and biomarkers

We found no significant differences between the two groups in terms of off-therapy period, age at recruitment, age at diagnosis, auxological parameters or markers of lipid and glucose metabolism. The hemostatic biomarker profile showed a statistically significant difference in ET-1 and HMW-AD levels between patients with 25-OHD < 20 ng/mL and patients with 25-OHD ≥ 20 ng/mL ($P = 0.035$ and 0.015, respectively) (Table 1).

Table 1 Clinical and biochemical characteristics of ALL patients according to 25-OHD levels

Variables	25-OHD		*P* value
	< 20 ng/mL	≥ 20 ng/mL	
Off-therapy period (mon)	26.4 ± 26.4	31.2 ± 19.2	0.58
Age at recruitment (y)	9.5 ± 4.3	10.1 ± 3.9	0.60
Age at diagnosis (y)	5.3 ± 4.0	5.5 ± 3.7	0.85
Height (SDS)	− 0.01 ± 0.96	− 0.27 ± 0.83	0.33
BMI (SDS)	0.89 ± 0.81	0.91 ± 1.06	0.94
HOMA-IR	2.6 ± 2.1	2.7 ± 1.8	0.82
Total cholesterol (mg/dL)	152 ± 23	149 ± 26	0.60
HDL cholesterol (mg/dL)	51 ± 10	49 ± 9	0.56
LDL cholesterol (mg/dL)	87 ± 17	86 ± 20	0.79
Triglycerides (mg/dL)	70 ± 34	66 ± 26	0.62
hsCRP (mg/L)	6.18 ± 13.0	3.66 ± 1.39	0.27
ET-1 (pg/mL)	1.96 ± 0.61	2.34 ± 0.59	*0.035*
HMW-AD (μg/mL)	5.12 ± 2.52	3.41 ± 2.00	*0.015*
TAT (μg/L)	3.91 ± 4.82	3.70 ± 3.76	0.86
vWFAg (%)	90.7 ± 19.5	89.0 ± 16.7	0.52
D-Dimers (ng/dL)	297.6 ± 152.4	363.9 ± 204.9	0.22
Fibrinogen (mg/dL)	265.6 ± 48.1	261.4 ± 36.8	0.73
FMD	10.5 ± 4.8	8.8 ± 3.8	0.20
C-IMT	0.48 ± 0.06	0.43 ± 0.06	*0.023*
APAO	10.0 ± 2.2	10.1 ± 1.8	0.85

A P value < 0.05 was considered statistically significant. *BMI* body mass index, *HDL-C* high-density lipoprotein-cholesterol, *LDL-C* low-density lipoprotein-cholesterol, *hsCRP* high sensitive C-reactive protein, *ET-1* endothelin-1, *HMW-AD* high molecular weight adiponectin, *TAT* thrombin–antithrombin complex, *vWFAg* von Willebrand factor antigen, *FMD* flow-mediated vasodilatation, *C-IMT* carotid intima-media thickness, *APAO* antero-posterior abdominal aorta diameter, *25-OHD* vitamin D

25-OHD deficiency and vascular ultrasound studies

The C-IMT was higher in patients with 25-OHD < 20 ng/mL than in patients with 25-OHD ≥ 20 ng/mL (0.48 ± 0.06 vs. 0.43 ± 0.06 mm, $P = 0.023$). No differences were found for the other vascular ultrasound parameters.

In multivariable additive regressions adjusting for the confounding effect of both BMI and LDL-C, the 25-OHD levels were related to responses ET-1, C-IMT, and HMW-AD. Increases in vitamin D levels (measured on a log-scale) were significantly associated with drops in mean levels of C-IMT (coefficient = − 0.035, $P = 0.0163$) and HMW-AD (coefficient = − 1.652, $P = 0.0008$), and rises in ET-1 mean levels (coefficient = + 0.326, $P = 0.0077$). None of the other factors among those listed in Table 1 were included in the final models, since they were not significantly associated with the responses.

Discussion

In this study, we found a higher prevalence of 25-OHD deficiency in ALL survivors compared to healthy controls. While some authors reported lower prevalence of vitamin D deficiency (29%) in survivors of childhood cancer [3], most of the literature suggests that ALL subjects may suffer from high vitamin D deficiency [1, 2, 24]. Our results agree with such findings.

In addition, we found a significant negative association between vitamin D levels and vascular function in survivors of childhood ALL. Patients with 25-OHD levels < 20 ng/mL showed a statistically significant thicker C-IMT. After adjusting for possible confounders, higher 25-OHD levels seemed to lead to a reduction in C-IMT.

The link between vitamin D and cardiovascular disease has been postulated in adulthood [25], but the role of vitamin D on cardiovascular homeostasis is still controversial [26]. Moreover C-IMT has been reported to be related to vitamin D levels in obese children and adolescents [27], and low vitamin D levels in childhood were associated with increased C-IMT in adulthood [28], suggesting that suboptimal childhood vitamin D levels should be considered a possible risk factor for adult cardiovascular disease. Furthermore vitamin D deficiency is definitely associated with increased C-IMT and a higher prevalence of carotid plaques according to a large pooled analysis of cohort studies [29].

Vitamin D has been encountered as an independent risk factor for cardiovascular disease. Endothelial dysfunction, a condition that may predict long-term cardiovascular disease, is mainly due to oxidative stress. There are some potential pathophysiological mechanisms linking low vitamin D levels with vascular function in ALL survivors. Vitamin D and its receptor VDR seem to modulate the oxidative stress

[30], and to protect endothelial cells via the modulation of apoptosis [31]. The oxidative stress, primarily resulting from the treatment for leukemia, might represent the trigger for endothelial dysfunction, enhanced by dietary and lifestyle changes, ultimately contributing to long-term metabolic and cardiovascular disease [32].

To the best of our knowledge, no previous study has evaluated the relationship between vitamin D and vascular function in childhood ALL survivors. However, considering together the reported data, the finding of a link between vitamin D and C-IMT in a population at risk of vascular dysfunction, as are ALL survivors, is definitely a crucial item to take into account in the management of this setting of patients.

Surprisingly, in addition to the negative association between 25-OHD and C-IMT, we also found out a positive association between 25-OHD and ET-1.

ET-1 is considered an important factor in the development of vascular dysfunction, acting as a vasoconstrictor and pro-inflammatory agent [33]. In endothelial dysfunction there is an increased expression of ET-1 in smooth muscle cells and in macrophages [34]. Hence, in this scenario a negative relationship could be expected and better explained.

Interestingly, in experimental studies, vitamin D induces a dual upregulation of ET-1 and nitric oxide, in a model of endothelial homeostasis [35]. Confirming this result, others found that 1,25-dihydroxyvitamin D3 significantly potentiated ET-1 signaling in an experimental model of human coronary artery cell culture [36].

Although limited by the small sample size, in this regard the result of a crosstalk between vitamin D and ET-1 might be an expression of endothelial homeostasis, rather than merely a casual finding. Moreover, we should consider that patients cured of childhood leukemia show higher ET-1 levels as compared to healthy subjects, as previously demonstrated [17].

To further confirm the influence of vitamin D deficiency on vascular and endothelial function is the significant direct link between 25-OHD and HMW-AD. Higher vitamin D levels correspond to higher HMW-AD levels, predictors of a better endothelial profile. In fact, HMW-AD has a protective role against cardiovascular derangement through the release of nitric oxide from the endothelium [37].

Our hypothesis is that in childhood ALL survivors, vitamin D interplays with the activation of endothelial biomarkers and endothelial dysfunction in a balance aiming to restore the compromised endothelial homeostasis. Based on the cross-sectional design, our study includes ALL survivors after ending treatment for leukemia, in clinical remission. Further studies, including children with ALL at the acute stage are needed to clarify this topic.

In conclusion, survivors of childhood leukemia have higher prevalence of vitamin D deficiency compared to healthy controls. This condition appears to be linked to vascular dysfunction as expressed by C-IMT and biomarkers. The relationship with endothelial biomarkers is complex and not yet clearly elucidated. Since vitamin D status is likely to be not only a clinical condition but rather a mediator of endothelial homeostasis, vitamin D balance should be carefully monitored in childhood ALL survivors characterized by high cardiovascular risk.

Author contributions MFF and NS contributed equally to the work. PM has drawn the design of the study, wrote the manuscript until its final version; VMR Muggeo analyzed the data and critically revised the article; PG took part in the conception and the design of the study and revised the article; MD gave contribution to the analysis of data and to the drafting of the manuscript; MA made the acquisition of laboratory data; CN contributed mainly in the acquisition of data and drafting the article; MMC made the acquisition and interpretation of cardiovascular data, he also revised the article; GD contributed to the data interpretation and manuscript revising; MFF participated in the design of the study and interpretation of data, very critically and carefully revised the paper; NS participated in the conception and design of the study and revised the article. All the authors approved the final version.

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Compliance with ethical standards

Ethical approval All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Conflict of interest The authors have no conflicts of interest to disclose. No financial or non-financial benefits have been received or will be received from any party related directly or indirectly to the subject of this article.

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