25-hydroxyvitamin D concentration in paediatric cancer patients from Scotland: A prospective cohort study

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Abstract

Children with cancer are potentially at high risk of plasma 25-hydroxyvitamin D [25(OH)D] inadequacy and despite UK vitamin D supplementation guidelines their implementation remains inconsistent. Thus, we aimed to investigate 25(OH)D concentration and factors contributing to 25(OH)D inadequacy in paediatric cancer patients. A prospective cohort study of Scottish children aged <18 years, diagnosed with and treated for cancer (patients) between Aug 2010-Jan 2014 was performed, with control data from Scottish healthy children (controls). Clinical and nutritional data were collected at defined periods up to 24 months. 25(OH)D status was defined by the Royal College of Paediatrics and Child Health (2013); inadequacy [<50 nmol/L: deficiency (<25 nmol/L), insufficiency (25-50 nmol/L)], sufficiency (51-75 nmol/L), optimal (>75 nmol/L). Eighty-two patients [median(IQR) age 3.9(1.9-8.8); 56% males)] and 35 controls [median(IQR) age (6.2(4.8-9.1); 49% males] were recruited. 25(OH)D inadequacy was highly prevalent in the controls (63%; 22/35), and in the patients (64%; 42/65) at both baseline and during treatment (33-50%). Non-supplemented children had the highest prevalence of 25(OH)D inadequacy at every stage with 25(OH)D median(IQR) ranging from 32.0 (21.0-46.5) nmol/L to 45.0(28.0-64.5) nmol/L. Older age at baseline [R=-0.46; p<0.001], overnutrition (BMI ≥85th centile) at 3 months [p=0.005; RR=3.1] and not being supplemented at 6 months (p=0.04; RR=4.3) may have contributed to lower plasma 25(OH)D. Paediatric cancer patients are not at higher risk of 25(OH)D inadequacy than healthy children at diagnosis; however prevalence of 25(OH)D inadequacy is still high and non-supplemented children have a higher risk. Appropriate monitoring and therapeutic supplementation should be implemented.

INTRODUCTION

Plasma 25-hydroxyvitamin D (25(OH)D) inadequacy (<50 nmol/L; deficiency and insufficiency) is a recognised health problem[1]. Despite vitamin D supplementation guidelines[2,3], their implementation remains inconsistent[4] and 25(OH)D inadequacy in healthy children ranges from 14% to 49% worldwide[5]. A recent systematic review reported prevalence of plasma 25(OH)D deficiency and insufficiency of 41% and 59% respectively in European paediatric cancer patients, higher than healthy children and paediatric cancer patients from North America (15% and 46%) and the Middle East (24% and 51%)[6].

Plasma 25(OH)D is primarily obtained from UVB sunlight through dermal synthesis, but it can also be obtained from the diet. However few foods naturally contain vitamin D[7], and in the UK fortification is rare[8]. In high latitude countries, like Scotland[8], populations are at an increased risk of 25(OH)D inadequacy. Other factors contributing to 25(OH)D inadequacy in children have been attributed to skin pigmentation, obesity and age (infants and adolescents)[2,7].

Children treated for cancer experience multiple side-effects, which might affect plasma 25(OH)D. These include phototoxicity, which requires avoidance of direct sunlight, reduced dietary intake[9], hepatotoxicity and nephrotoxicity, which may interfere with the activation of 25(OH)D[10]. 25(OH)D inadequacy in children increases risk of bone fractures, rickets and slow growth[11], with a subsequent increased risk of osteoporosis [12]. Most children and adolescents treated for cancer survive into adulthood[13], but have an increased risk of developing metabolic syndrome, cardiac complications and have a reduced peak bone mass[13]. Despite the importance of vitamin D to health, the high prevalence of 25(OH)D inadequacy in Europe and the recent call for high-quality population-based longitudinal cohort studies, there are few published studies in the UK and none in Scotland, investigating plasma 25(OH)D concentration in paediatric cancer

patients[6]. To address this clinical question we aimed to: investigate both plasma 25(OH)D and parathyroid hormone (PTH) concentration of paediatric cancer patients at defined time points for 24 months; compare plasma 25(OH)D concentration of healthy children with a paediatric cancer cohort from Scotland and explore possible factors (age, ethnicity, gender, seasonality, nutritional status, diagnosis, treatment and the use of nutritional support) contributing to plasma 25(OH)D inadequacy at baseline and at 3 and 6 months.

METHODS

Study design, population and time-line

A prospective cohort study was performed. Eligibility criteria were: children aged <18 years; diagnosed with cancer (ICCC-3)[14] or Langerhans Cell Histiocytosis between Aug-2010 and Jan-2014; attending the South East Scotland regional centre (56ºN) for Haematology and Oncology at the Royal Hospital for Sick Children (RHSC), Edinburgh or Ninewells Hospital, Dundee and patients were recruited consecutively. We excluded children who were treated palliatively at any time. Children were monitored for a maximum period of 24 months and all measurements were obtained at baseline (newly diagnosed), 3, 6, 9 and 12 months and every 6 months thereafter. Factors contributing to plasma 25(OH)D inadequacy were only explored at baseline and at 3 and 6 months due to the reduced sample size at later stages.

Anonymised control data were obtained from the control subjects recruited within a case-control study of Vitamin D in children with epilepsy carried out between July 2013 and March 2014 at RHSC. Controls were recruited over an overlapping time frame, similar representative seasons and regions as the cancer patients. Consecutive potentially eligible controls attending the RHSC Emergency Department (which serves SE Scotland) who were previously healthy, not in

extremis nor had an existing chronic condition (and specifically no epilepsy or other seizure disorder) and who required blood samples to be taken as part of their clinical assessment (eg child with a fever), were invited to the epilepsy study. Participants to the epilepsy study along with their parents gave written informed consent and where appropriate – informed assent. Recruitment was completed when the target sample size for each season was achieved. Advice on vitamin D supplementation was not provided prior to sample collection. Ethical approval for secondary use of the anonymised control data for comparison to that of the cancer patients in this study, without the need for additional consent, was given by the South East Scotland Research Ethics Service. Control data were not matched for age, sex or BMI; however samples were matched for synthesising (1st of April-30th Sep) and non-synthesising periods (1st Oct-31st Mar) for comparative reasons.

Demographics and clinical parameters

Clinical data (diagnosis, treatment protocol and length of treatment) and demographic data (age, gender, ethnicity and socioeconomic deprivation) were collected from medical notes. Treatment intensity was classified according to Kazak et al.[15] As a proxy marker for socioeconomic deprivation of individuals, we used Standard Index of Multiple Deprivation (SIMD).[16]

The paediatric cancer cohort was grouped according to the wider definition of solid tumours, haematological cancers, brain tumours and other associated diagnoses.

Data collection

Plasma 25(OH)D, parathyroid hormone (PTH), calcium, phosphate and magnesium concentrations were measured. Plasma 25(OH)D was analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) technique at the Royal Infirmary of Glasgow and PTH was analysed using the Immulite 2000 Intact PTH technique at the Royal Infirmary of

Edinburgh. The immediate coefficient of variation (%) for the assays were ≤8.9% and 5.7% respectively. Calcium, phosphate and magnesium were analysed using the Abbott Architect c8000 at RHSC

Plasma 25(OH)D concentration was classified as synthesising (1st of April-30th Sep) and non-synthesising periods (1st Oct-31st Mar). Plasma 25(OH)D was defined according to the Royal College of Paediatrics and Child Health (2013)[2]; deficiency (<25 nmol/L), insufficiency (25-50 nmol/L), sufficiency (51-75 nmol/L), optimal (>75 nmol/L). Plasma 25(OH)D inadequacy was used when 25(OH)D concentration was <50 nmol/L. Plasma 25(OH)D toxicity was defined as >175 nmol/L (with associated symptoms) and the PTH reference as 1.7-7.5pmol/L[17].

Height (or length) and weight were measured using standard procedures. Body mass index (BMI) centile was calculated and UK BMI growth centiles were used. Nutritional status was classified as underweight (BMI ≤2.3th centile), healthy weight (BMI >2.3rd to <85th centile) and overweight (BMI ≥85th centile)[18]. Vitamin D intake was assessed using a 24 hour multi-pass recall method[19] to establish patterns of change in vitamin D throughout the study period. This was analysed in WinDiets® (Univation Ltd 2005) programme[20]. Any nutritional treatment and vitamin D supplementation was recorded. Nutritional treatment was prescribed according to Subjective Global assessment by the multidisciplinary team and consisted of enteral +/- parenteral nutrition (macronutrient) and micronutrient (vitamin D according to UK RCPCH guidelines[2] or multivitamins), and a combination of macronutrients and micronutrients.

This study was granted ethical approval from NHS Scotland (NHS REC 06-51104-52).

Statistical analyses

The Statistical Package for Social Science (IBM-SPSS for Windows Statistics, version 19) was employed to analyse all data. Descriptive statistics were used to evaluate the prevalence of

plasma 25(OH)D inadequacy. Comparisons between the paediatric cancer cohort and the healthy controls were performed using Mann-Whitney; correlations between plasma 25(OH)D and the following variables: calcium, PTH, BMI centile and age, were performed using Spearman's correlation. Univariate associations between demographic data and categorical variables were established by $\chi 2$-test. P <0.05 was considered statistically significant. We followed the STROBE guidelines for the presentation of our data[21]. No a priori sample size estimation was performed for this pilot study in a regional cohort of paediatric cancer patients.

RESULTS

Demographic and clinical characteristics

Thirty-three of 35 healthy controls and 65 of 82 paediatric cancer patients had plasma 25(OH)D samples available at baseline (figure 1). Of the healthy controls, two (6%) samples were never returned due to laboratory issues. Demographic and clinical characteristics of the population are presented in table I and II. Gender, ethnicity and socioeconomic status as well as age at diagnosis did not statistically differ between groups. BMI centiles were significantly lower in the paediatric cancer cohort. Twenty-four treatment protocols were used to treat the paediatric cancer cohort, the median (IQR) time follow up was 312 (123.5-653.2) days and 22% (n=18) were classified as low risk, 37% (n=30) as medium risk and 41.5% (n=34) as high risk. The time between diagnosis and baseline measurements was 15.5 (10.0-25.0) days and between the start of cancer treatment and baseline measurements was 9.5 (6.0-19.5) days. All patients were receiving cancer treatment when plasma 25(OH)D samples were taken.

Plasma 25(OH)D concentration

At baseline, of the 82 paediatric cancer patients, 17 (21%) did not have plasma 25(OH)D available due to clinical reasons (figure 1), 34 (41%) were obtained during the synthesising period and 31 (38%) during non-synthesising period. There was no difference [U (453); p=0.3] between the synthesising (median 39.0, IQR 30.0-62.0) and non-synthesising period (median 36, IQR 16.0-61.0) in plasma 25(OH)D concentration in the cancer cohort at any time-point, apart from the 3 month follow up (figure 2). Of the 35 controls, 19 (54%) were obtained during the synthesising period and 12 (34%) during the non-synthesising period. Plasma 25(OH)D (nmol/L) statistically differed [U (42.5); p=0.003)] during the non-synthesising (median 26.0, IQR 18.0-46.5) and synthesising period (median 56.5, IQR 45.5-78.0) . Baseline plasma 25(OH)D of the cancer cohort did not differ from the healthy controls (p=0.7).

At baseline, prevalence of plasma 25(OH)D inadequacy was 64% (42/65) in cancer patients and 63% (22/35) in healthy children. There was a higher prevalence of plasma 25(OH)D deficiency in paediatric cancer patients (n=19; 29%) in comparison with healthy children (n=8; 22%) but this was not statistically significant (p=0.2; $\chi 2$-test). In the cancer cohort, prevalence of plasma 25(OH)D inadequacy ranged between 33-50% throughout the study period (figure 3). Patients with solid tumours had the highest prevalence of 25(OH)D inadequacy (34%) followed by haematological malignancies (26%) at both baseline (table II) and at all time-points (supplemental table I). At baseline, of 32 solid tumour patients 37.5% (n=12) were deficient and 31.2% (n=10) were insufficient and of 26 haemalogical malignancy patients 19.2% (n=5) were deficient and 46.1% (n=12) were insufficient (table II).

Nutritional support was prescribed to 26% (21/82) of paediatric cancer patients at baseline of which, 14/82 (17%) were on macronutrient (enteral +/- parenteral nutrition), and 7/82 (8%) were on both macronutrient (enteral+/- parenteral nutrition) and micronutrient. The median (IQR) time

between the start of nutritional support and baseline was 8 (0-23) days. Eighty percent (66/82) of cancer patients received vitamin D from one or more forms of nutritional support for several days or weeks during the study period. Of these, 39/82 received macronutrient supplementation providing 292 (128-332)IU per day, 48/82 (58%) received both micronutrient and macronutrient supplementation providing 464 (440-664)IU per day and 21/82 (26%) received macronutrient only and micronutrient (+/- macronutrient) supplementation. The vitamin D intake from diet alone was 68 (24-76)IU per day and supplementation of vitamin D ranged from 400IU per day to 20,000 IU single dose of vitamin D during the study period.

Paediatric cancer patients who were not supplemented had the lowest plasma 25(OH)D. The prevalence of plasma 25(OH)D inadequacy stratified by nutritional support and stages of disease is presented in table III. This was highest in children who did not receive supplementation ranging from 32.0 (21.0-46.5) nmol/L at 18 months to 45.0 (28.0-64.5) nmol/L at 24 months. In contrast, paediatric cancer patients supplemented with micronutrient (+/- macronutrient) had the lowest prevalence of plasma 25(OH)D inadequacy and the highest plasma 25(OH)D at most stages ranging from 63.0 (42.7-128.5) nmol/L at 6 months to 82.0 (57.0-128.5) nmol/L at 12 months. This was followed by children supplemented with macronutrient only, which ranged from 43.0 (29.2-75.7) nmol/L at baseline to 79.0 (49.0-93.0) nmol/L at 6 months. A considerable number of patients in the macronutrient subgroup had already received micronutrient supplementation. Of the 7 patients who were on macronutrient support at 6 months, all of them had received micronutrient supplementation previously. Likewise, 2/5 (40%) patients on macronutrient support at 12 months and 1/2 (50%) patients at 18 months had received micronutrient supplementation in the previous follow up. Moreover, micronutrient supplementation was significantly associated with lower prevalence of plasma 25(OH)D

inadequacy (Fisher's Exact test; p=0.04; RR 0.27; 95% CI 0.04-1.8) at 6 months. Three patients reached plasma 25(OH)D concentration of >175nmol/L[17] following a single high dose (20,000 IU/day) of vitamin D.

Plasma 25(OH)D did not correlate with plasma calcium, phosphate, magnesium and PTH at any stage in the cancer cohort, however PTH and plasma 25(OH)D concentration correlated in the healthy controls [r=0.6; p<0.001].

Factors contributing to 25(OH)D inadequacy concentration at baseline and at 3 and 6 months of treatment

Age negatively correlated with plasma 25(OH)D concentration in paediatric cancer patients [r=-0.46; p<0.001], only at baseline, and in healthy children [r=-0.42; p<0.02], whereby older children had lower plasma 25(OH)D concentration. Although, BMI centile was not significantly correlated with plasma 25(OH)D concentration in the paediatric cancer cohort at baseline [r=-0.2; p=0.08], 3 months [r=-0.2; p=0.2] and 6 months [r=-0.2; p=0.3], and in the healthy control [r=-0.3; p=0.3], overnourished paediatric cancer patients were more likely to have higher prevalence of plasma 25(OH)D inadequacy [χ^2-test(8.3); df(1); p=0.005; RR 3.1; 95% CI 1.4-14.0] at 3 months than healthy and undernourished children with cancer, regardless of whether the patients were on nutritional supplementation. Non-supplemented children were more likely to have inadequate plasma 25(OH)D concentration (RR 4.3; 95% CI 1.1-4.7) at 6 months (Fisher's Exact test; p=0.04) compared with those supplemented with micronutrients.

None of the following categorical variables were significantly associated with plasma 25(OH)D status and paediatric cancer patients at any stage; treatment risk, diagnostic criteria, ethnicity and gender.

DISCUSSION

This is the first study investigating plasma 25(OH)D concentration at diagnosis and during treatment in paediatric cancer patients from Scotland. Our results show a high prevalence of plasma 25(OH)D inadequacy during the study period. Plasma 25(OH)D concentration in paediatric cancer patients and age matched healthy controls were similar; however, our paediatric cancer cohort showed no seasonal variation. Children diagnosed with solid tumours exhibited the lowest plasma 25(OH)D concentration and the only effective method to achieve optimal plasma 25(OH)D concentration was by supplementing with vitamin D. Only 3 factors, and each at 1 time point only, contributed to plasma 25(OH)D inadequacy; older age was the only factor at baseline, overnutrition at 3 months and not being supplemented at 6 months during treatment.

Prevalence of plasma 25(OH)D

In contrast to North England[22] but in agreement with a recent Scottish small study [24], our study shows that plasma 25(OH)D concentration in newly diagnosed paediatric cancer patients and healthy children were comparable suggesting that patients from Scotland are not at higher risk of plasma 25(OH)D inadequacy than healthy children at diagnosis. However, these concentrations are lower than those reported in paediatric cancer patients from Europe[6]. Of note, 11% of paediatric cancer patients were on vitamin D supplementation at baseline, which may have contributed to higher plasma 25(OH)D concentration at this stage. Additionally, there was a higher representation of winter samples in the healthy controls than the paediatric cancer cohort (30% v 43%), which might have contributed to the unexpectedly higher prevalence of vitamin D inadequacy in the healthy controls.

Optimal plasma 25(OH)D in children is essential to allow optimal growth, calcium homeostasis and skeletal development[3]. Children treated for cancer may have impaired growth velocity during treatment[27], which can also be exacerbated by vitamin D inadequacy. Current UK guidelines on vitamin D are aimed at healthy children and stipulate that children under 5 years old should be supplemented with 7.5-10µg/day (300-400 IU) of vitamin D[2]. We have clearly established that most cancer patients who were not supplemented were either deficient or insufficient, or eventually became deficient as shown by the high prevalence of plasma 25(OH)D inadequacy (33-50%). Furthermore, macronutrient supplementation alone prevented plasma 25(OH)D inadequacy, but patients rarely reached optimal concentration, suggesting that macronutrient supplementation, which is fortified with vitamin D, does not meet the requirements for vitamin D in this population. Finally, vitamin D supplementation taken in the form of multivitamins or as therapeutic supplementation was essential to achieve optimal 25(OH)D concentration in all paediatric cancer patients. Remarkably, we found that older children were at higher risk of plasma 25(OH)D inadequacy at baseline and therefore would also require supplementation, which is not stipulated in the RCPCH (2013) guidelines[2]. However, it is important to note that three patients on single high dose (20,000 IU) vitamin D supplementation reached 25(OH)D >175 nmol/L concentration[17]. Therefore, we recommend vitamin D supplementation for all paediatric cancer patients, but emphasise the need for close monitoring to avoid potential toxicity.

Unlike healthy children, our paediatric cancer cohort did not show any seasonal variation in plasma 25(OH)D concentration. These findings are supported by a study performed in survivors of childhood cancer from the USA (latitude 34ºN)[26] but contrasts with two studies[22,25]; one performed in North England (latitude 54.9ºN) during and after therapy[22] and the other

performed in Israel (latitude 31ºN) in paediatric cancer patients during therapy[25]. Therefore, we hypothesise that Scottish paediatric cancer patients are not exposed to enough sunlight during the summer months, probably due to the multiple treatment side-effects[26,27], and that diet alone is insufficient to replenish plasma 25(OH)D stores.

Stratification of the data by diagnosis revealed results consistent with a recent systematic review[6] and a large study (n=2198) performed in the adult oncology population from USA. Patients diagnosed with solid tumours had prevalence of plasma 25(OH)D inadequacy of 71% and 75% respectively, our study showed that children diagnosed with solid tumours exhibited the highest prevalence of plasma 25(OH)D inadequacy (69%; deficiency 37.5% and insufficiency 31.2%) at diagnosis. However, our study also showed high prevalence of plasma 25(OH)D inadequacy in the haematological malignancy group (65%; deficiency 19.2% and insufficiency 46.1%) at diagnosis, which contrasts with findings from elsewhere[6,26]. Although others have reported similar prevalence of vitamin D inadequacy in Canadian children diagnosed with haematological malignancies[29,30], the measured vitamin D was 1,25-dihydroxyvitamin D (1,25(OH)2D), which is not equivalent to plasma 25(OH)D. Despite supplementation with macronutrients and micronutrients, prevalence of 25(OH)D inadequacy remained high during treatment for solid tumours and haematological malignancies highlighting the need for more rigorous monitoring at all stages.

In line with recent evidence[6,22], our study found a relationship between PTH and plasma 25(OH)D in healthy controls, but not in paediatric cancer patients. Although, in health PTH measured alongside plasma 25(OH)D is considered the most sensitive physiological measure of plasma 25(OH)D status and bone homeostasis[31], our study suggests that there might be other factors influencing their relationship. We were unable to investigate this due to the relatively

small sample; however it has been attributed to the type of cancer and the different treatments, including chemotherapy and corticosteroids, which can lead to nephrotoxicity and hepatotoxicity, in turn interfering with the 25(OH)D, 1,25(OH)2D and PTH metabolism[10,32]. Furthermore, a stronger relationship between plasma PTH and 25(OH)D develops with age[33] which might have affected our results, since the controls were slightly older.

Factors contributing to reduced plasma 25(OH)D concentration at baseline and at 3 and 6 months

Consistent with a meta-analysis[6], older age was associated with reduced plasma 25(OH)D concentration in paediatric cancer patients at baseline. This association was also found in our healthy controls, in line with a study performed in healthy children from the USA[34], which could reflect the widespread issue of vitamin D. Teenagers tend to eat less vitamin D rich foods, especially fortified foods, and spend less time playing outdoors than younger children[24]. Additionally, the high levels of vitamin D inadequacy during treatment could have been attributed to the fact that patients were supplemented with a very low dose of vitamin D (440-664 IU). A higher dose of 600IU is recommended for all paediatric patients (including infants), whilst therapeutic doses are age dependent and all doses are over 1000IU per day[3,17]. Alongside infancy, puberty is accompanied by a rapid period of growth and appropriate plasma 25(OH)D concentration are essential to allow for optimal growth[35]; thus this population should be targeted and appropriate doses should be prescribed to all patients.

Like healthy individuals[7], but contrary to other studies investigating factors contributing to plasma 25(OH)D inadequacy in paediatric cancer patients[24,25], our results showed that overnourished children maybe more likely to have plasma 25(OH)D inadequacy following 3

months of treatment and this was regardless of nutritional support. An inverse relationship between high BMI and plasma 25(OH)D in the healthy population is well established[7], which has been attributed to a reduction in plasma 25(OH)D availability due to the sequestration of vitamin D by adipose tissue[36]. Overweight children require higher doses of chemotherapy and glucocorticoids than normal weight or undernourished children. Additionally, cancer treatments tend to be most intense during the first 3 to 6 months post-diagnosis. Chemotherapy agents commonly used in cancer treatment can cause hepatotoxicity and nephrotoxicity and thus inhibit the activation of vitamin D[29]. Whilst glucocorticoids stimulate vitamin D catabolism and can increase the risk of vitamin D deficiency [29]. Therefore, higher doses of chemotherapy agents and glucocorticoids may explain this association between overnourished patients and lower 25(OH)D concentration

Limitations of the study and future research

The reduced sample size at later stages of the study precluded considering factors associated with plasma 25(OH)D at later stages of treatment. Some cancer patients were already on nutritional support at baseline, which could potentially have affected plasma 25(OH)D concentration. It should be noted that although age did not statistically differ between the controls and the cancer cohort, the controls were slightly older. Also, the higher proportion of samples obtained from the non-synthesising period in the controls may have distorted the high plasma 25(OH)D inadequacy reported. Finally, there were only 2 non-Caucasian patients (dark skin) in both groups, which could explain why lower plasma 25(OH)D concentration was not associated with ethnicity. Future research should include large multicentre epidemiological studies that are better able to identify factors contributing to plasma 25(OH)D inadequacy in the different types of cancer

during treatment. Also, randomised controls trials in which the effects of vitamin D supplementation on clinical outcome, particularly bone mass density, are warranted.

CONCLUSION

We have highlighted that Scottish paediatric cancer patients have a high prevalence of plasma 25(OH)D inadequacy at diagnosis and during treatment and that older age, not being supplemented and possibly being overnourished potentially contributes to inadequacy. Importantly, we recommend vitamin D supplementation to all paediatric cancer patients given that macronutrient supplementation alone prevented further 25(OH)D inadequacy, but rarely produced optimal concentration, and high longitudinal inadequacy rates continued throughout the study.

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Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

Contributor's Statement Page

Raquel Revuelta Iniesta: designed the study, collected the data from the paediatric cancer cohort, analysed the data, drafted the manuscripts and provided final approval of the manuscript.

Ilenia Paciarotti: collected the data from the paediatric cancer cohort, provided critical feedback and final approval of the manuscript.

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Mark Brougham: supervised the study and data collection from the cohort, provided critical feedback and final approval of the manuscript.

David Wilson: designed, coordinated and supervised the study, provided critical feedback and final approval of the manuscript.

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Tables

Table I. Characteristics of the paediatric cancer population and the healthy controls.

Baseline characteristics	Paediatric cancer cohort		Controls		P value
Total sample (n)	82		35		
Age median (IQR)	3.9 (1.9-8.8)		6.2 (4.8-9.1)		0.1[1]
BMI centile median (IQR)	50 (19.0-84.5)		60.5 (43.7-89.5)		0.003[1]
Plasma 25(OH)D median (IQR)	38.0 (21.0-61.0)		37.5 (23.0-58.0)		0.7[1]
	n	%	n	%	
Gender					0.5[2]
male	46	56.1	17	48.6	
female	36	43.9	18	51.4	
Ethnicity					0.6[2]
White	80	97.6	33	94.3	
Non-white	2	2.4	2	5.7	
SES					0.06[2]
I	15	18.3	3	8.6	
II	13	15.8	8	22.9	
III	15	18.3	5	14.3	
IV	24	29.3	5	14.3	
V	15	18.3	14	40.0	
Haematological malignancies	**35**	**43**	-	-	
ALL	29	35			
AML	3	4			
CML	2	2			
HLH	1	1			
Solid tumours	**39**	**47**			
Lymphomas	10	12			
Neuroblastoma	6	7	-	-	
Retinoblastoma	2	2	-	-	
Renal tumours	6	7	-	-	
Hepatic tumours	1	1	-	-	
Malignant bone tumours	4	5	-	-	
Soft tissue sarcoma	5	6	-	-	
Germ cell tumours	1	1	-	-	
Malignant epithelial neoplasm	4	5	-	-	
Others unspecified malignancy	0	0	-	-	
Other associated diagnoses	**3**	**4**	-	-	
LCH	3	4			
Brain tumours-CNS tumours	**5**	**6**			

ALL: acute lymphoblastic leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; HLH: haemophagocytic lymphohistiocytosis; LCH: Langerhan's Cell Histiocytosis; CNS: central nervous system; [1]Mann-Whitney test; [2]chi square-test. SIMD: Standard Index of Multiple Deprivation presented as a quintile where "I" denotes the most deprived and "V" the least deprived.

Table II. Plasma 25(OH)D concentration of the paediatric cancer cohort and the healthy controls at baseline.

		N	Median (IQR)	Deficient N (%)	Insufficient N (%)	Sufficiency N (%)	Optimal N (%)	P value
controls		n=35	37.5 (23.0-58.0)	8 (22.8)	14 (40.0)	6 (17.1%)	5 (14.3)	0.06[1]
Paediatric cancer	**Cases**	n=65	38.0 (21.0-61.0)	19 (29.2)	23 (35.4)	16 (24.6)	7 (10.8)	0.01[1]
	Diagnostic group	ST n=32	35.0 (16.0-60.0)	12 (37.5)	10 (31.2)	8 (25.0)	2 (6.25)	0.02[1]
		HM n=26	38.0 (27.7-52.2)	5 (19.2)	12 (46.1)	6 (23.1)	3 (11.5)	0.04[1]
		BT n=5	69 (14.5-75.5)	2 (40.0)	0	2 (40.0)	1 (20.0)	-
		OAD n=2	80	0	0	1 (50.0)	1 (50.0)	-
	Nutrition Support	None n=44	34.0(20.2-52.7)	16 (36.4)	15 (34.1)	11 (25.0)	3 (6.9)	0.006[1]
		Macronutrients n=14	43.0(29.2-75.7)	2 (14.3)	6 (42.8)	3 (21.4)	3 (21.4)	0.8[2]
		Micronutrients n=7	71.0(41.0-97.0)	1 (14.3)	1 (14.3)	3 (42.9)	2 (28.6)	0.9[2]

ST: Solid tumours; HM: Haematological malignancies; BT: Brain tumours; OAD: other associated-diagnoses; [1]χ^2-test; [2]Fisher's Exact test; 25(OH)D reference ranges: Deficiency: <25 nmol/L; Insufficiency: 25-50 nmol/L; sufficiency: 51-75 nmol/L; optimal: >75 nmol/L; 25(OH)D inadequacy (<50 nmol/L).

Table III. Prevalence of plasma 25(OH)D inadequacy with data stratified by nutritional support and at different stages of the disease

Time line	Nutritional support		Deficiency		Insufficiency		Sufficiency		Optimal		Median (IQR)
		N	N	%	N	%	N	%	N	%	
Baseline N=65	None	44	16	25	15	23	11	17	2	3	34.0(20.2-52.7)
	Macronutrients	14	2	3	6	9	3	5	3	5	43.0(29.2-75.7)
	Micronutrients+/- macronutrients	7	1	1	1	1	3	5	2	3	71.0(41.0-97.0)
3 months N=55	None	25	4	7	11	20	6	11	4	7	45.0(32.0-56.0)
	Macronutrients	9	1	2	1	2	4	7	3	5	67.0(48.0-76.5)
	Micronutrients+/- macronutrients*	20	1	2	6	11	7	13	6	11	67.5(38.0-87.7)
6 months N=34	None	9	3	9	2	6	4	12	0	0	45.0(16.5-68.0)
	Macronutrients	7	0	0	2	6	1	3	4	12	79.0(49.0-93.0)
	Micronutrients+/- macronutrients	18	1	3	3	9	4	12	10	29	78.0(49.2-134.5)
9 months N=30	None	16	1	3	7	23	6	20	2	7	45.0(31.0-61.0)
	Macronutrients	8	1	3	2	7	2	7	3	10	59.0(35.5-84.25)
	Micronutrients+/- macronutrients	6	0	0	0		2	7	4	13	77.5(66.5-101.0)
12 months N=24	None	11	3	12	4	17	1	4	3	12	36.0(24.5-79.0)
	Macronutrients	5	0	0	1	4	4	17	0	0	63.0(51.0-63.0)
	Micronutrients+/- macronutrients	8	0	0	0	0	5	21	3	12	82.0(57.0-128.5)
18 months N=18	None	12	3	17	7	39	2	11	0	0	32.0(21-46.5)
	Macronutrients	2	0	0	0	0	1	6	1	6	67.5(56.0-)
	Micronutrients+/- macronutrients**	3	0	0	0	0	3	17	0	0	64.0(42.7-134.0)
24 months N=12	None	5	1	8	2	17	2	17	0	0	45.0 (28.0-64.5)
	Macronutrients	0	0	0	0	0	0	0	0	0	
	Micronutrients+/- macronutrients	7	2	17	1	8	0	0	4	33	67.0(23.7-106.2)

[1]OAD: other associated diagnoses; 25(OH)D reference ranges: Deficiency: <25 nmol/L; Insufficiency: 25-50 nmol/L; sufficiency: 51-75 nmol/L; optimal: >75 nmol/L; 25(OH)D inadequacy (<50 nmol/L)

Supplementary material

Supplemental table I. Prevalence of plasma 25(OH)D inadequacy of paediatric cancer patients with data stratified by diagnostic criteria.

Figure legends

Figure 1. Flow chart showing the sample size at different stages of the study period

Figure 2. Plasma 25(OH)D with data stratified according to seasonal variation

Error bars are standard deviations; *$p<0.05$, independent t-test used to compare 25(OH)D concentration between synthesising (1st of April-30th Sep) and non-synthesising periods (1st Oct-31st Mar).

Figure 3. Plasma 25(OH)D concentration (left) and prevalence of 25(OH)D deficiency and insufficiency (right) at different stages of the study period.

Error bars (left) are standard deviations.