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ORIGINAL ARTICLE

# Vitamin D deficiency and its correction in children with sickle cell anaemia

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**Abstract** Vitamin D deficiency is common in sickle cell anaemia (SCA, HbSS), although its significance and optimal means of correction are unknown. We conducted an audit to assess the clinical significance of 25-hydroxy vitamin D (25-OHD) deficiency in children with SCA and to evaluate two methods of vitamin D supplementation. We audited 25-OHD levels in 81 children with SCA and looked for statistical associations with biochemical, haematological and clinical parameters. In a separate group of regularly transfused children with SCA, we compared changes in 25-OHD blood concentrations following treatment with either high-dose intramuscular ergocalciferol ($n$=15) or 4 days of high-dose oral cholecalciferol ($n$=64). Ninety-one percent of children with SCA had 25-OHD levels <20 μg/L. The 25-OHD levels were negatively correlated with increasing age ($P$<0.001) but showed no significant relationship to laboratory measurements, transcranial Doppler velocities or hospital attendance. Both intramuscular ergocalciferol and oral cholecalciferol supplementations resulted in increases of 25-OHD blood concentration to normal levels. The mean dose of ergocalciferol was greater than that of cholecalciferol (7,729 versus 5,234 international units (IU)/kg, $P$<0.001), but the increment in 25-OHD levels was significantly greater in the oral cholecalciferol group (6.44 versus 2.82 (ng/L)/(IU/kg), $P$<0.001). Both approaches resulted in vitamin D sufficiency for about 120 days. Increased 25-OHD concentration was significantly associated with increased serum calcium concentration. Vitamin D deficiency is very common in SCA and can be effectively corrected with high-dose intramuscular ergocalciferol or 4 days of high-dose oral cholecalciferol. Prospective, randomised studies are needed to assess the clinical value of vitamin D supplementation.

**Keywords** Cholecalciferol · Ergocalciferol · Alkaline phosphatase · Serum calcium · Vitamin D · Sickle cell disease

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## Introduction

Sickle cell disease (SCD) is a common and severe genetic disorder. Although the mutation in the beta globin gene is only expressed in the erythron, a cascade of pathological events flows from the altered rheological properties of blood. Almost all tissues are affected including bones; bony pain is common during acute vaso-occlusion, and chronic bone and joint damage occurs with increasing age. Therefore, vitamin D deficiency might mimic some symptoms of SCD or exacerbate clinical complications of the condition [1].

Vitamin D is implicated in a wide range of physiological processes beyond bone metabolism. It is involved in the control of blood pressure, insulin secretion and lipid metabolism, and deficiency has been linked to the development of stroke, heart failure, renal impairment, immunodeficiency and cancer [2]. Vitamin D deficiency is increasingly diagnosed and may reach epidemic frequencies in many countries; it is probably most significant in paediatric populations [3]. Severe deficiency in children causes rickets and osteomalacia, with characteristic bony deformation, and is relatively rare. Subclinical deficiency may only be detected on blood testing but has been linked to impaired bone and muscle development, reduced growth velocity, immunodeficiency and bone pains.

All these are particularly relevant to children with SCD and may exacerbate or mimic symptoms of that condition [4].

As the majority of vitamin D in humans results from sun exposure, the incidence of deficiency would be expected to vary with latitude, season and skin pigmentation. Several studies from different areas have documented low vitamin D levels in adults and children with SCD over the last few years. For example, a study in St Louis (USA) found that 64 % of children with SCD had severe vitamin D deficiency (<10 ng/mL) and only 2.2 % had sufficient vitamin D (>30 ng/mL) [5]. Similarly, a study in Madrid (Spain) found that only 21 % of children with SCD had sufficient vitamin D [6], and a study from Paris (France) found that 76 % of children were deficient [7]. Although low vitamin D levels are very common in these studies, in most cases, the studies identify subclinical deficiency, detected on blood testing but without obvious clinical effects, such as bone disease or increased complications of SCD. It is much less clear if this subclinical deficiency is associated with any adverse effects and how it should be treated, if at all. This is particularly important in SCD which is associated with many pathological processes which could be exacerbated by vitamin D deficiency, including bone disease, vasculopathy, immunodeficiency and renal impairment [8]. To a large extent, this reflects uncertainty about vitamin D deficiency in the general population and the lack of consensus on what constitutes deficiency. A recent study suggests that vitamin D-binding protein is lower in Black Americans than white, and currently used normal ranges may be inappropriately high for black populations [9]. Similarly, there is no clear evidence on when and how to offer vitamin D supplements [4].

We have routinely monitored vitamin D levels for about 7 years in children with SCD and tried several different therapeutic approaches including daily low-dose treatment, very high-dose intramuscular treatment with ergocalciferol (vitamin $D_2$) (stoss treatment) [10] and short courses of high-dose cholecalciferol (vitamin $D_3$). Because of concern about the effects of iron overload and chelation on bone health, we monitor children with SCD receiving regular blood transfusions particularly closely, with monthly measurement of vitamin D levels and supplementation when deficiency occurs. We have audited this routinely collected data to answer two questions: (1) Is vitamin D deficiency clinically significant in children with SCD? (2) Can deficiency be effectively corrected with a short course of high-dose vitamin D in this population?

## Methods

### Patients and setting

King's College Hospital is a teaching hospital in South London (UK) with a specialist paediatric SCD clinic. Steady state blood tests are taken once per year, and measurements include 25-hydroxy vitamin D (25-OHD), corrected serum calcium and alkaline phosphatase levels. Results from a cross section of 81 patients with sickle cell anaemia (SCA, HbSS) were used to assess the prevalence of vitamin D deficiency and its pathophysiological correlates. These patients all had 25-OHD measurements in 2006 before we routinely corrected vitamin D deficiency, and had not been prescribed vitamin D supplements. The effects of vitamin D supplementation could not be assessed in this group because 25-OHD levels were not routinely measured following treatment. Routinely collected laboratory and transcranial Doppler (TCD) velocities were analysed. Days spent in hospital and accident and emergency attendances were analysed for 2006, to assess possible clinical effects. All hospital attendances were included, although the vast majority of admissions were for direct complications of SCA. The study was an audit of clinical practice and was approved by institutional audit committee. All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008 (5).

Approximately 30 children with SCA receive regular blood transfusions every 3 to 4 weeks, mostly for primary and secondary stroke prevention. The 25-OHD levels are measured monthly in these children, and vitamin D supplements prescribed when levels were less than 20 μg/L. The effects of vitamin D supplementation were analysed in these children because of the monthly measurements, allowing comparison between different replacement strategies over time. Various strategies have been used to correct vitamin D deficiency in these patients, and data were analysed from this group to assess and compare the efficacy of different replacement strategies.

### Vitamin D supplementation

Three strategies were used for vitamin D supplementation in the transfused children:

1. Four hundred international units (IU) ergocalciferol oral daily as part of multivitamin supplementation. This was used initially but abandoned because 25-OHD levels failed to increase significantly or even fell. Anecdotally, children and parents reported poor adherence with this approach because of the unpleasant taste of multivitamins and the need for treatment on a daily basis over a long time.
2. A single dose of ergocalciferol (300,000 IU) injected intramuscularly and repeated when 25-OHD <20 μg/L. This was used routinely from 2008 to 2010, subsequently depending on patient's preference.
3. High-dose oral cholecalciferol, 20,000 IU (weight <30 kg) or 40,000 IU (weight >30 kg) daily for 4 days only, repeated when 25-OHD <20 μg/L. This has been used routinely since 2010.

### Vitamin D assays

Serum total vitamin D was measured by enzyme immunoassay on the Liaison semi-automated machine (DiaSorin, Inc., Stillwater, MN, USA). Performance of the assay was determined by regular quality control samples, and the between- and within-batch coefficient of variation of values was less than 12 %.

### Analysis

Statistical analysis was performed using SPSS version 21 (IBM, Portsmouth, UK). Analysis included tests for normal distribution, correlation, independent sample *t* tests and paired sample *t* tests. *P* values <0.05 were considered significant. Relevant laboratory parameters for males and females are given in Table 1.

## Results

### Distribution of 25-OHD levels

Vitamin D levels were normally distributed (one-sample Kolmogorov-Smirnov test 0.152). The mean 25-OHD level in cross-sectional analysis was 12.8 μg/L (standard deviation 5.3) with a range of 4.4 to 31.9. There was no significant difference in levels between the 46 boys and 35 girls (12.9 versus 12.6 μg/L, $P=0.77$). One (1.2 %) child had sufficient 25-OHD (>30 μg/L), six (7.4 %) had vitamin D insufficiency (20–30 μg/L), and 74 (91 %) were classified as deficient (<20 μg/L).

### Correlation of 25-OHD levels

The 25-OHD levels were correlated with the following parameters: age, haemoglobin, white cell count, corrected calcium, alkaline phosphatase, lactate dehydrogenase levels and time-averaged mean of the maximum velocity on transcranial Doppler scanning (TAMMX) (Table 2). All parameters were normally distributed (Kolmogorov-Smirnov test >0.05), apart from serum calcium which was analysed after logarithmic transformation. Only age correlated with 25-OHD levels significantly, with vitamin D levels falling with increasing age. The correlation between 25-OHD levels and the logarithm of corrected serum calcium levels approached significance ($P=0.054$). The total white cell count correlated positively with 25-OHD levels with borderline significance.

The number of days in hospital for a year varied from 0 to 24, with a mean of 1.4 and a median of 0.66; 81.5 % of children had no admissions during the year. There was no significant difference in the 25-OHD levels of children with no days in hospital compared to those who spent 1 day or more (12.8 versus 12.9 μg/L, $P=0.939$). The number of accident and emergency (A&E) attendances varied from 0 to 4 in the year, with a mean of 0.27 and a median of 0.63; 77.8 % of children had no A&E attendances. As for hospital days, there was no significant difference in 25-OHD levels between those who did not attend A&E and those who attended one or more time (12.9 versus 12.4 μg/L, $P=0.734$).

### Intramuscular ergocalciferol

Eleven different children were treated on 15 separate occasions with 300,000 IU of ergocalciferol in this audit. The mean dose was 7,729 IU/kg, with a range of 4,225 to 16,667. The mean 25-OHD level increased from 6.1 to 27.9 μg/L ($P<0.001$, paired *t* test), measured after a median of 33 days (range 19–83). All children initially had vitamin D deficiency; following treatment, six (40 %) still had deficient levels, three (20 %) were classified as having insufficiency, and six (40 %) had vitamin D sufficiency. The mean increase in 25-OHD per unit of ergocalciferol per kilogram was 2.8 ng/L/unit/kg with a range of 0.92 to 5.49. The 25-OHD levels stayed elevated above deficient levels (>20 μg/L) for a median of 122 days

**Table 1** Descriptive data of basic laboratory data on the 81 children analysed in the cross-sectional part of the study

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | *N* | Mean | Standard deviation | *N* | Mean | Standard deviation |
| Age (years) | 46 | 9.8 | 4.4 | 35 | 9.7 | 3.9 |
| Haemoglobin (g/dL) | 35 | 7.7 | 1.3 | 32 | 7.9 | 1.2 |
| White cell count ($\times 10^9$/L) | 35 | 11.7 | 3.2 | 32 | 11.7 | 3.5 |
| Corrected calcium (mmol/L) | 46 | 2.27 | 0.14 | 34 | 2.30 | 0.06 |
| Alkaline phosphatase (IU/L) | 46 | 173 | 51 | 34 | 183 | 45 |
| Lactate dehydrogenase (IU/L) | 34 | 593 | 129 | 31 | 565 | 126 |
| TAMMX (cm/s) | 42 | 118 | 32 | 32 | 132 | 25 |

*TAMMX* time-averaged mean of the maximum velocity on transcranial Doppler scanning

**Table 2** Correlation between 25-OHD levels, corrected serum calcium and other biomarkers of disease severity in children with sickle cell anaemia

| | Number | Pearson correlation | Significance (2-tailed) |
|---|---|---|---|
| Age (years) | 81 | −0.409 | <0.001 |
| Haemoglobin (g/dL) | 67 | −0.007 | 0.95 |
| Reticulocyte count ($\times 10^9$/L) | 66 | 0.123 | 0.323 |
| White cell count ($\times 10^9$/L) | 67 | 0.236 | 0.054 |
| Ln (serum calcium) (mmol/L) | 80 | 0.216 | 0.054 |
| Alkaline phosphatase (IU/L) | 80 | −0.066 | 0.559 |
| Lactate dehydrogenase (IU/L) | 77 | 0.160 | 0.202 |
| TAMMX (cm/s) | 77 | 0.136 | 0.237 |

All variables were normally distributed apart from serum calcium, which was logarithmically transformed before analysis

(range 27–259). Alkaline phosphatase levels did not change significantly with ergocalciferol injection (from 166 to 171 IU/L, $P$=0.498), but calcium levels increased from a mean of 2.18 to 2.23 mmol/L ($P$=0.010). This change is not biologically significant, but indicative of a biochemical effect. There were no adverse events or side effects recorded.

### Oral cholecalciferol

Twenty-eight different children were treated with high-dose oral cholecalciferol on 64 separate occasions. The mean dose was 5,234 IU/kg, with a range of 2,105 to 9,722. The 25-OHD levels increased from a mean of 11.3 to 45 μg/L ($P$<0.001, paired $t$ test), measured after a median of 24 days (range 14–70). Following treatment, 3 (4.6 %) children still had deficient 25-OHD levels, 6 (9.3 %) had insufficiency, and 53 (83 %) had vitamin D sufficiency. The mean increase in 25-OHD levels per unit of cholecalciferol per kilogram was 6.4 ng/L/unit/kg (range −0.4 to 16.3). The 25-OHD levels stayed elevated above deficient levels (>20 μg/L) for a median of 112 days (range 23–282). After oral cholecalciferol, mean alkaline phosphatase levels fell significantly (220 to 213 IU/L, $P$=0.028) and mean serum calcium increased (2.23 to 2.26 mmol/L, $P$<0.001). Again, these changes are not biologically significant but indicate biochemical activity of the cholecalciferol.

Oral cholecalciferol resulted in a significantly greater increase in 25-OHD levels, with the increment nearly double that of ergocalciferol per dose per kilogram. However, there was no significant difference between the number of days of vitamin D sufficiency following treatment, with both forms of treatment keeping 25-OHD >20 μg/L for a median of about 120 days (Table 3).

**Table 3** Comparison of treatment with intramuscular ergocalciferol ($n$=15) and oral cholecalciferol ($n$=64)

| | Ergocalciferol (intramuscular) | Cholecalciferol (oral) | $P$ |
|---|---|---|---|
| Mean dose of vitamin D (IU/kg) | 7,729 | 5,234 | <0.001 |
| Mean increment plasma 25-OHD (μg/L) | 21.8 | 34.0 | 0.020 |
| Mean increment plasma 25-OHD per dose per kg ((ng/L)/(IU/kg)) | 2.82 | 6.44 | <0.001 |
| Median days of 25-OHD >20 μg/L after dose | 122 | 112 | 0.476 |

Mean dose, increment and increment/kilogram were compared using independent sample $t$ test; median days >20 μg/L were compared using independent sample Kruskal-Wallis test

## Discussion

As with other studies, we found a high prevalence of vitamin D deficiency in our cross-sectional survey of children with SCA, with 91 % of children classified as being deficient. The reasons for these low levels are likely to include the latitude in London providing relatively little strong sunlight, the dark skin of most of the patients and dietary intake. It is theoretically possible that nephropathy related to SCA may also have impaired the ability of subjects in this audit to metabolise vitamin D, although no children had evidence of significant renal impairment [11].

However, it is difficult to know the significance of low 25-OHD levels and whether there is any pathological association. In our cross-sectional analysis of 81 patients, there was no suggestion that low vitamin D levels were associated with increased episodes of acute pain, as indicated by days spent in hospital or A&E attendances. Our study had limited power to detect any such effect because the majority of patients had vitamin D deficiency. A study of 53 children in the USA found significantly lower 25-OHD levels in children with chronic pain compared to those without [11], and the same authors subsequently showed an improvement in chronic pain in a single patient with severe vitamin D deficiency causing osteomalacia following high-dose replacement therapy [12]. However, no other studies have demonstrated a significant link between 25-OHD levels and acute pain, including a study of 139 children in the USA and UK [5]. The same study did find an association between 25-OHD levels and pulmonary function, but not with the rate of acute chest syndrome [5]. Perhaps the best evidence that 25-OHD levels are of clinical significance in SCA comes from two studies, one in the USA and one in Saudi Arabia, which both noted a high incidence of low bone mineral density in adults with SCA and vitamin D deficiency [13, 14].

Low vitamin D levels have been identified in many studies in non-SCA populations [3], and there is similar uncertainty about the significance of biochemical vitamin D deficiency in various clinical settings. There is general consensus that treatment is appropriate in the presence of skeletal manifestations such as rickets and osteopenia [15], although there is less agreement about the value of vitamin D supplements for other reasons [16]. In children with SCA, we have routinely prescribed vitamin D supplements in the presence of biochemical deficiency (<20 μg/L), because of concern about coexistent bone disease, potential implications for immune function [17] and vascular endothelial function [18], particularly in those children with cerebrovascular disease receiving regular blood transfusions. There are many different formulations of vitamin D supplements, with no accepted replacement regime. The current recommendation from the British National Formulary for Children recommends oral replacement daily for 8 weeks in symptomatic vitamin D deficiency [16]. We have tried various regimes involving daily supplements for many weeks and found them all to be unsuccessful due to limited adherence, possibly related to unpleasant taste. We are also particularly keen to avoid prolonged oral therapy, which may distract from prophylactic penicillin and reduce compliance with this essential medication in SCD [19].

We found that both high-dose oral cholecalciferol and intramuscular ergocalciferol resulted in significant increases in 25-OHD levels when measured about 4 weeks later. The 25-OHD levels remained in the sufficient range for a median of about 120 days, and this did not differ significantly between oral and intramuscular routes. The large increase in 25-OHD was accompanied by a small but statistically significant increase in serum calcium levels on paired *t* test, but with no change in serum alkaline phosphatase levels. Oral cholecalciferol resulted in a two to three times greater increment in 25-OHD levels than intramuscular ergocalciferol when doses were adjusted for the weight of the patient (Table 3). One explanation for this could be the known difference in absorption and assimilation of vitamin D for oral as opposed to intramuscular administration—in that oral bolus doses result in more replenishment of tissue stores [20]. Another possible explanation for the greater increment with cholecalciferol could be related to the vitamin D assay reacting less specifically with ergocalciferol (vitamin $D_2$) and therefore underestimating total vitamin D levels in those treated with ergocalciferol.

No children suffered any significant side effects or documented hypercalcemia. It was not possible to formally assess patient's preferences as part of this audit. Both high-dose oral and intramuscular approaches seemed to be well tolerated. Anecdotally, some children preferred intramuscular treatment, and 95 % of children prescribed oral cholecalciferol showed an increment in 25-OHD levels of at least 10 μg/L, suggesting that adherence to oral vitamin D was high. Previous studies have compared the efficacy of cholecalciferol and ergocalciferol and oral versus intramuscular routes of administration. The overall greater potency of cholecalciferol has been confirmed in most other studies and a meta-analysis [21].

This retrospective audit has shown that vitamin D levels are low in the majority of children with SCA, although it is not clear how clinically significant this is and what benefits arise from correcting deficiency. In theory, increasing serum calcium levels could increase erythrocyte dehydration in SCA and precipitate vaso-occlusion [22]. We have routinely corrected vitamin D deficiency in transfused children and found that both high-dose intramuscular and oral administrations increase levels effectively for about 3 months.

This study is limited by its retrospective nature and the lack of data on changes bone density and health, making it hard to assess the value of vitamin D supplementation. Both cholecalciferol and ergocalciferol are effective, with the former showing greater potency. Prospective trials of vitamin D replacement are needed in SCD to assess the clinical value of such treatments as high-dose oral cholecalciferol.

**Conflict of interest** The authors have no conflicts of interest to disclose.

**Ethical standards** The study was an audit of clinical practice and was approved by institutional audit committee. Individual patient consent was not necessary as the audit involved the analysis of data which were all collected as part of routine clinical care. All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008 (5).

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