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Vitamin D Insufficiency and Liver Iron Concentration in Transfusion Dependent Hemoglobinopathies in British Columbia

Hatoon M. Ezzat[1,3,*], *John Wu*[2,3], *Heather McCartney*[2,3], *Heather A. Leitch*[1]

[1] Hematology, St. Paul's Hospital, University of British Columbia, Vancouver, BC, Canada

[2] Hematology Oncology, BC Children's Hospital, University of British Columbia, Vancouver, BC, Canada

[3] Inherited Bleeding and Red Blood Cell Disorder Program of British Columbia, University of British Columbia, Vancouver, BC, Canada

Corresponding Author & Address:

Hatoon M. Ezzat[*]

Hematology Division, St. Paul's Hospital, University of British Columbia, 490-1144 Burrard Street, Vancouver, BC V6Z 2A5, Canada; Phone: 604-684-5794; Fax: 604-684-5705; Email: hezzat@providencehematology.com

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Keywords: Thalassemia Major, Liver Iron Concentration, 25 hydroxyvitamin D, transfusional iron overload

ABSTRACT

Patients with thalassemia major (TM) and other chronically transfused hereditary anemias are at increased risk of complications including endocrinopathies and bone disease due to iron overload. Vitamin D is important for bone health. Vitamin D deficiency is common in patients with transfusional iron overload, and the mechanism remains unclear. The first step in vitamin D metabolism, hydroxylation, occurs in the liver and liver iron overload may interfere with this step. This study investigates an association between degree of liver iron overload and vitamin D levels in patients with transfusion dependent hemoglobinopathies. Patients with TM, hemoglobin Eβ TM (Eβ TM), and congenital dyserythropiotc anemia (CDA) attending the Inherited Bleeding and Red Blood Cell Disorder Program in British Columbia (IBRBCD BC), Canada were identified. Included patients had an assessment of liver iron concentration (LIC) by MRI and endocrinology assessment including 25 hydroxy vitamin D level. Thirty patients were identified. The mean LIC was 5.13 mg/g dry weight (DW). Vitamin D deficiency/insufficiency was identified in 19 (63.3%). Eleven (36.7%) patients had an LIC ≥5 mg/gDW, 8 of whom had a vitamin D level <60 nmol/L, indicating moderate vitamin D insufficiency or deficiency. There was a significant association between LIC >5 mg/gDW and vitamin D level <60nmol/L (P= 0.027) and there was a significant inverse correlation between LIC and vitamin D (R=-0.33). These results indicate an association between increased LIC and vitamin D insufficiency/deficiency, suggesting that liver iron overload may indeed affect vitamin D metabolism. Prospective trials are needed to confirm these results.

INTRODUCTION

Thalassemia major (TM) is the most common hereditary anemia worldwide. Over the past decade the natural history of TM has evolved from a therapeutic focus on preventing mortality to eliminating morbidity. However, bone health remains one of the most common concerns in the care of TM patients. The underlying etiology of bone disease is multifactorial. Vitamin D deficiency has been noted in both transfusion and non-transfusion dependent thalassemic patients and the mechanism is unclear [1, 2]. Vitamin D has been demonstrated in previous studies as crucial for bony health in patients with hemoglobinopathies [3, 4]. Vitamin D deficiency results in decreased bone mineralization and increased cortical bone loss, leading to osteopenia

osteoporosis syndrome (OOS). Hypogonadotrophic hypogonadism (HH) has been demonstrated to be the major cause of OOS in TM, but other contributing factors may co-exist such as vitamin D deficiency and desferrioxamine use, which may affect bone metabolism by inhibiting proliferation of osteoblast-like cells [5] and by causing bone dysplasia [6].

Neither vitamin D3 nor vitamin D2 are biologically active after being absorbed through the gastrointestinal tract or skin. In the liver vitamin D3 undergoes hydroxylation by the cytochrome P-450-dependent enzyme 25-hydroxylase to produce the major circulating form, 25 hydroxy vitamin D (25-OH D3). Hepatic dysfunction may theoretically affect the metabolism of vitamin D. 25-OH D3 is the best indicator of vitamin D status in the body, as it reflects both vitamin D produced by the skin and obtained from food and supplements [7]. The steps of vitamin D metabolism are illustrated in Figure 1.

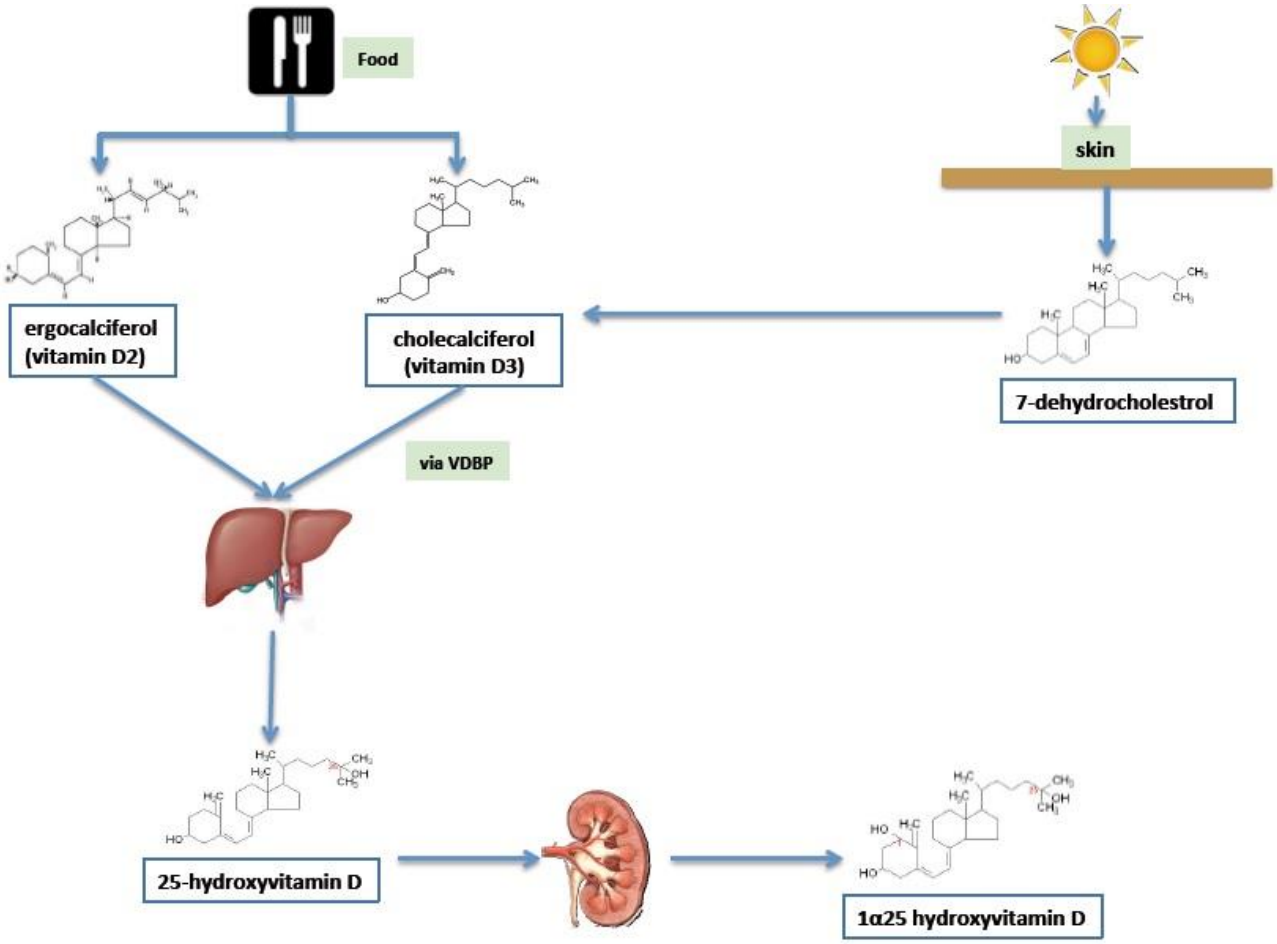

Figure 1: Vitamin D enters the body through dietary intake (10-20%) and endogenous synthesis in the skin (80-90%) after exposure to ultraviolet light from the sun. Food contains both cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) (Henry, 2011). Neither vitamin D3 nor vitamin D2 are biologically active, for this they must be metabolized to the hormonally active 1α25-hydroxy vitamin D3 (1α25-OH D3). Vitamin D3 is released into the circulation and transported to the liver by vitamin D-binding protein (VDBP) a protein synthesized and secreted by hepatocytes. In the liver vitamin D3 undergoes hydroxylation by the cytochrome P-450-dependent enzyme (25-hydroxylase) to produce the major circulating form, 25-OH D3. The final step of vitamin D synthesis is the production of 1α25-OH D3, the hormonally active form of vitamin D, in the kidney.

Hepatic osteodystrophy (HO) is a complex of structural and metabolic changes resulting in alterations in bone mineral metabolism in which vitamin D hydroxylation in the liver is affected. Studies suggest that there is a higher incidence of HO in cholestatic liver disease, and that serum 25-OH D3 continues to decrease as cirrhosis develops [8]. Chow et al showed in 15 patients with hereditary hemochromatosis that removal of excess iron by therapeutic venesection produced a significant increase in the mean serum 25-OH D3 level [9]. Vitamin D deficiency and cardiac iron

overload [10] has been examined in hemoglobinopathies patients with transfusional iron overload, and cardiac iron overload was more prevalent in patients with vitamin D deficiency. There are few studies, however, examining a possible association between vitamin D deficiency and hepatic iron overload. As the liver is the major site of hydroxylation of vitamin D, it is possible that liver iron overload may lead to vitamin D deficiency. The objective of this study was to examine a possible association between vitamin D insufficiency and deficiency and the severity of liver iron loading and to determine whether vitamin D hydroxylation may be affected by the presence of iron overload in the liver.

METHODS AND MATERIALS

Patients with transfusion dependent hemoglobinopathies including TM, Eβ thalassemia (Eβ TM), and Congenital Dyserythropiotic Anemia (CDA) were identified through a search of the Inherited Bleeding and Red Blood Cell Disorder Program of British Columbia (IBRBCD BC) program database in the adult and pediatric programs. Patients included were those who had an assessment of liver iron concentration (LIC) done by MRI (T2* or R2*) and underwent endocrinology assessment including 25-OH D3 levels between the 1st of January 2009 and the 31st of December 2011. Patient charts were reviewed and clinical data abstracted. Statistical analysis was performed using SPSS for Windows, version 19.0. Descriptive analysis was used to summarize patient characteristics. Continuous variables were described by means and medians while categorical variables were expressed by frequency. Association between variables was determined by Chi-square or Fisher's Exact analyses, where appropriate. A two-sided P value of <0.05 was considered statistically significant. Pearson correlation coefficient was performed using SPSS.

There is no consensus on what levels of vitamin D constitute insufficiency or deficiency [11, 12, 13, 14, 15]. Commonly used definitions include: normal 25-OH D3, >75 nmol/L; insufficiency, 50-75 nmol/L; deficiency, <50 nmol/L and severe deficiency, <25 nmol/L. Due to small numbers of patients with levels <50 nmol/L in this study, for our purposes we divided the group with vitamin D insufficiency into; mild insufficiency, 60-75 nmol/L; and moderate insufficiency, 50-59 nmol/L; and the remainder of the categories were as above. LIC values were: LIC <2 mg/gmDW, normal; >2 but <5 mg/gmDW, mild; >5 but <7 mg/gmDW, moderate; and >7 mg/gmDW, severe hepatic iron overload [16]. Osteopenia was defined as a Z score <-1.5 while <-2.5 indicated osteoperosis.

We examined whether there was an association between serum ferritin 1000 mcg/L or more and vitamin D level. We then examined a possible association between LIC and vitamin D level. Clinical variables such as age, type of chelation, ethnicity, amount of annual blood transfusion and bone mineral density (BMD) were tested for a possible association with levels of 25-OH D3.

This analysis was done in accordance with requirements of the Institutional Research Ethics Boards at St. Paul's and BC Children's Hospitals.

RESULTS

Clinical, Laboratory and Radiological Characteristics

Of 206 patients with hemoglobinopathies registered in the adult or pediatric IBRBCD programs, 30 transfusion dependent hemoglobinopthy patients had both LIC and vitamin D levels available. Clinical, laboratory and radiological characteristics are shown in Table 1. All patients were transfused on a two to four weekly interval to maintain a pre transfusion hemoglobin between 90-100 g/L. The mean amount of blood received annually was 9633.9 mls/year (n=26); four patients had missing transfusion data.

Twenty seven (90%) patients were receiving vitamin D supplementation with a dose ranging from 400 to1000 IU daily. All patients were receiving iron chelation therapy (Table 1). None of the patients were receiving cardiac or liver medications such as beta-blockers or interferon. One patient had Wilson's disease (LIC 3 mg/gDW) while none of the other patients had chronic liver disease from other causes and hepatitis B, C and Human Immunodeficiency Virus (HIV) serologies were negative in all patients.

Table 1: Clinical, Laboratory and Radiological characteristics of 30 patients with transfusion dependent hemoglobinopathies

Characteristics	N (%)
Age	16 – 51 (mean 23 years)
Gender	
Male	14 (46.6)
Female	16 (53.3)
Diagnosis	
β TM	21 (70)
Eβ TM	6 (20)
CDA	3 (10)
Ethnicity	
Asian	12 (40)
Indian	8 (26.7)
Others[1]	6 (20)
Middle Eastern	3 (10)
Caucasian	1 (3.33)
African	0
Vitamin D Supplement	
Y	26 (86.7)
N	4 (13.3)
Calcium Supplement	
Y	26 (86.7)
N	4 (13.3)
Type of iron chelation	
Desferasirox	18 (60)
Desferrioxamine	9 (30)
Combination[2]	3 (10)
Deferiprone	0

Laboratory Data	n	Minimum	Maximum	Mean	Std. deviation
Ferritin (mcg/L)	30	223	3553	1182.8	702.93
25-OH D3 (nmol/L)[3]	30	18	125	67.6	28.6
<75	19				
<60	13				
<25	2				
Ca^{2+} (mmol/L)[4]	30	2.2	2.52	2.34	0.08
PO^{4} (mmol/L)[4]	30	0.78	1.74	1.28	0.26
ALP (U/L)[4]	30	48	494	125.1	84.477

Laboratory Data	n	Minimum	Maximum	Mean	Std. deviation
LIC (mg/gDW)[5]	29	1.1	31.8	5.13	5.75
≥7	5				
5-7	3				
2-5	16				
<2	5				
Cardiac IC (mS)[6]	30	13	74.8	37.39	11.98
Z-score L1-4[7]	30	-4.7	-0.1	-2.3	1.25
Z-score hip[7]	30	-4.1	-0.1	-1.57	1.02

- [1] unable to determine
- [2] desferrioxamine + deferiprone n=1, desferrioxamine + desferasirox n=2
- [3] Value ranges of 25-OH D3 (nmol/L): severe deficiency, <25 nmol/L; mild to moderate deficiency, 25-74 nmol/L; optimal levels, 75-200 nmol/L; potential for vitamin D toxicity, >250 nmol/L
- [4] Normal ranges: Ca2+ 2.22-2.67 mmol/L, PO4 0.87-1.52 mmol/L, serum ferritin 15-130 mcg/L, ALP 40-130U/L
- [5] LIC: severe iron overload, >7 mg/gDW; moderate, 5-6.9 mg/gDW; mild, 2-4.9 mg/gDW; normal, <2mg/gDW
- [6] Cardiac iron concentration: normal iron concentration >30 mS
- [7] BMD: osteoporosis is 2.5 standard deviations or more below the average value for young healthy women (a T-score of <-2.5 SD) (Based on WHO criteria)

Associations and Correlations

There was no significant association between a serum ferritin level >1000 mcg/L and 25-OH D3<50nmol/L, < 60 nmol/L or < 75 nmol/L (P=NS for both). There was, however, a significant association between 25-OH D3 level <60 nmol/L

and LIC >5 mg/gDW (P=0.027). Moreover, there was an inverse correlation between LIC and 25-OH D3 level (correlation coefficient R= - 0.33). There was a trend toward an association between 25-OH D3 level <60 nmol/L and LIC >7 mg/gDW (P=0.064). There was no association between vitamin D level <60 nmol/L and either LIC <2 mg/gDW or LIC <5mg/gDW. These results are shown in Figure 2. Finally, vitamin D level correlated significantly with ALT (R= - 0.42; P<0.05) and AST (R= - 0.46; P<0.05), see Figure 3.

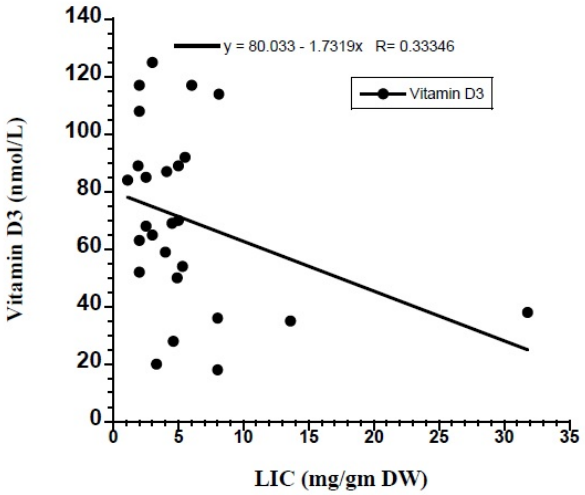

Figure 2: Correlation between LIC and vitamin 25-OH-D3

Comparing a yearly transfusion requirement of >220 mL/kg versus <220 mL/kg, ferritin level >1000 mcg/L showed a trend toward association with the higher transfusion requirement (P=0.06). There was no significant association between transfusion requirement and LIC of >7 or >5 mg/g DW, nor with vitamin D level of <75, <60 or <50 nmol/L.

The type of chelation had no association with vitamin D level <75 nmol/L or <60 nmol/L. Similarly, age had no association with LIC at any level analyzed, nor on vitamin D at any level. There was no significant association between ethnicity and vitamin D level. Finally, there was no association between degrees of OOS in relation to LIC (P=NS for all).

DISCUSSION

This study is a retrospective, cross-sectional review, which addresses the prevalence of vitamin D insufficiency and deficiency in patients with transfusion dependent hemoglobinopathies in a single center. It identifies an association between moderate vitamin D insufficiency/deficiency and the presence of liver iron overload. Specifically, of 30 patients, 13 (43.3%) had a 25-OH D3 level <60 nmol/L and only 2 (7%) had a normal level (Table 2), indicating that vitamin D deficiency/insufficiency was prevalent in our patient population despite supplementation in the majority (90%), in keeping with the results of prior analyses [17].

Table 2: Association between LIC and 25-OH D level <60 nmol/L; numbers of patients in each group

	LIC		Total
	< 5 mg/gDW	**≥ 5 mg/gDW**	
25-OH D < 60 nmol/L	5	8[1]	13
25-OH D ≥ 60 nmol/L	13	3	16
Total	18	11	

- [1] P=0.027

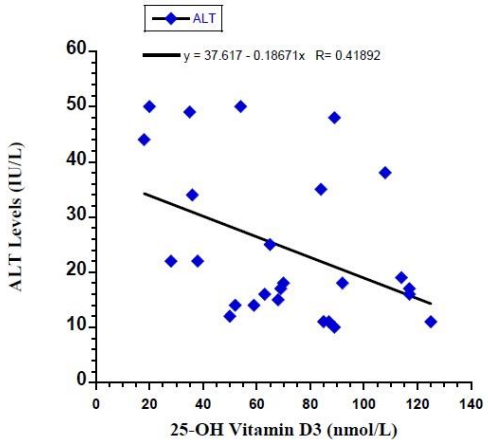

Figure 3 (a): Correlation between ALT and vitamin 25-OH-D3

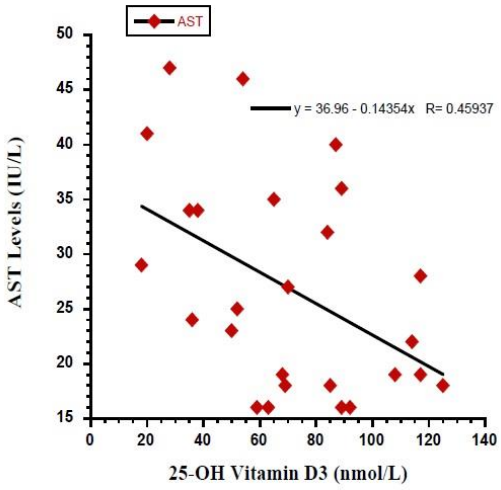

Figure 3 (b): Correlation between AST and vitamin 25-OH-D3

Only 5 patients (17%) had a normal liver iron concentration as shown in table 2. We found a significant association between vitamin D level

<60 nmol/L and LIC ≥5 mg/gDW (P=0.027) with a significant inverse correlation between LIC and vitamin D level (R=-0.33). In addition, there was a significant correlation between vitamin D and ALT (R=-0.418) and AST (R=-0.45). In the comparison between vitamin D level and LIC >7 mg/gDW (n=5, 17%), there was only a trend toward statistical significance, which may be a matter of sample size. Similarly, there may exist an association between severe vitamin D deficiency (< 25nmo/l, n=2, 7%) that was not recognized in this study due to small numbers. The finding of a significant association and correlation between vitamin D deficiency and the presence of moderate hepatic iron overload support exploration and validation of this effect in larger numbers and comparison to controls from the non-transfused population.

Serum ferritin level has been reported to correlate with LIC in this patient population [18]. In this analysis, there was no significant association between low vitamin D level and serum ferritin level >1000 mcg/L. This may reflect the myriad of factors that influence the ferritin level, or may be a matter of small sample size. However, it is notable that in non-transfusion dependent hemoglobinopathies (NTDT) such as thalassemia intermedia (TI), the ferritin level grossly underestimates the LIC, and vitamin D level may prove in future to be a simpler and more convenient indicator of abnormal LIC requiring intervention, particularly in centres with restricted access to imaging techniques or liver biopsy. This is potentially relevant not only to disorders such as TI, but also to other hemoglobinopathies, particularly given the lack of association between serum ferritin level and LIC in this study (P=NS). Given the importance of normal serum vitamin D levels and protection against bone disease in hemoglobinopathy patients, this result may indicate that ferritin level is not an ideal marker of hepatic iron overload and that intensification of chelation targeting a normal LIC may be appropriate; this presupposes that liver iron overload affects the first step of vitamin D hydroxylation in the liver and that offloading liver iron will improve this process. There was no significant association between the degree of transfusion requirement and elevated LIC, nor with low vitamin D level, which may indicate that iron chelation therapy compensated for higher transfusion requirement in terms of controlling LIC and therefore vitamin D level.

There was no significant association between ethnicity and BMD or vitamin D levels in this analysis, contrary to previously reported results [19, 20]. However, this finding may be a matter of limited numbers in each group. Similarly, there was no association between other clinical or laboratory features collected and analyzed and either vitamin D level or LIC, suggesting that confounding factors were not prevalent and that the association between vitamin D level <60 nmol/L and LIC >5 mg/gDW and inverse correlation between LIC and vitamin D, and correlation between ALT, AST and vitamin D levels, may be real findings that are possibly clinically relevant. These results, if verified in prospective trials, may help to identify patients requiring intensification of vitamin D and/or calcium supplementation, and/or intensification of iron chelation therapy in order to reduce the incidence of clinical consequences of vitamin D deficiency.

CONCLUSION

Vitamin D regulates calcium homeostasis and plays a major role in bone health. Our patients with transfusional iron overload had a high a prevalence of vitamin D deficiency/insufficiency (63.3%), in keeping with prior analyses [17]. We found a significant association between liver iron overload (LIC >5mg/g DW) and moderate vitamin D insufficiency/deficiency (<60 nmol/L), suggesting that liver iron overload may affect vitamin D metabolism and possibly increase the risk of clinical endpoints related to vitamin D insufficiency/deficiency. This is to our knowledge the first report of an association between vitamin D deficiency and elevated LIC, with an inverse correlation between the two parameters. This retrospective analysis should be considered hypothesis generating and should be verified in prospective analyses.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

[1] Vogiatzi MG, Macklin EA, Trachtenberg FL, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Fleisher M, Grady RW, Peterson CM, Giardina PJ; Thalassemia Clinical Research Network. Differences in the prevalence of growth, endocrine and vitamin D abnormalities among the various thalassemia syndromes in North America. *Br J Haematol.* **2009;** 146: 546-56. http://dx.doi.org/10.1111/j.1365-2141.2009.07793.x

[2] Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pourzahedgilani N, Bouzari N, Habibzadeh M, Hashemi R, Shamshirsaz AA, Aghakhani S, Homayoun H, Larijani B. Metabolic and endocrinologic complications in beta-thalassaemia major: A multicenter study in Tehran. *BMC Endocr Disord.* **2003;** 3: 4. http://dx.doi.org/10.1186/1472-6823-3-4

[3] Chatterjee M. Vitamin D and genomic stability. *Mutat Res.* **2001;** 475: 69-87. http://dx.doi.org/10.1016/S0027-5107(01)00080-X

[4] Holick M. Vitamin D and sunlight: strategies for cancer prevention and other health benefits. *Clin J Am Soc Nephrol.* **2008;** 3: 1548-54. http://dx.doi.org/10.2215/CJN.01350308

[5] Naves Díaz ML, Elorriaga R, Canteros A, Cannata Andía JB. Effect of desferrioxamine and deferiprone (L1) on the proliferation of MG-63 bone cells and on phosphate alkaline activity. *Nephrol Dial Transplant.* **1998;** 13: 23-8. http://dx.doi.org/10.1093/ndt/13.suppl_3.23

[6] Chan YL, Li CK, Pang LM, Chik KW. Desferrioxamine-Induced long bone changes in thalassaemic patients- Radiographic features, prevalence and relations with growth. *Clin Radiol.* **2000;** 55: 610-4. http://dx.doi.org/10.1053/crad.2000.0492

[7] Ohyama Y, Yamasaki T. Eight cytochrome P450 catalyze vitamin D metabolism. *Front Biosci.* **2004;** 1: 3007-18. http://dx.doi.org/10.2741/1455

[8] Dibble JB, Sheridan P, Hampshire R, Hardy GJ, Losowsky MS. Osteomalacia, vitamin D deficiency and cholestasis in chronic liver disease. *Q J Med.* **1982;** 51: 89-103.

[9] Chow LH, Frei JV, Hodsman AB, Valberg LS. Low serum 25-hydroxyvitamin D in hereditary hemochromatosis: relation to iron status. *Gastroentrology.* **1985;** 88: 865-9.

[10] Wood JC, Claster S, Carson S, Menteer JD, Hofstra T, Khanna R, Coates T. Vitamin D deficiency, cardiac iron and cardiac function in thalassaemia major. *Br J Haematol.* **2008;** 141: 891-4. http://dx.doi.org/10.1111/j.1365-2141.2008.07135.x

[11] Belhoul KM, Bakir ML, Saned MS, Kadhim AM, Musallam KM, Taher AT. Serum ferritin levels and endocrinopathy in medically treated patients with β thalassemia major. *Ann Hematol.* **2012;** 91: 1107-14. http://dx.doi.org/10.1007/s00277-012-1412-7

[12] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society. Evaluation, Treatment and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab.* **2011;** 96: 1911-30. http://dx.doi.org/10.1210/jc.2011-0385

[13] Holick MF. Vitamin D Deficiency. *N Engl J Med.* **2007;** 357: 266-81. http://dx.doi.org/10.1056/NEJMra070553

[14] Kwiatkowski JL. Management of transfusional iron overload – differential properties and efficacy of iron chelating agents. *J Blood Med.* **2011;** 2: 135-49. http://dx.doi.org/10.2147/JBM.S13065

[15] Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassemia major. *Br J Haematol.* **2000;** 110: 971-7. http://dx.doi.org/10.1046/j.1365-2141.2000.02298.x

[16] Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology.* **1986;** 6: 24-9. http://dx.doi.org/10.1002/hep.1840060106

[17] Napoli N, Carmina E, Bucchieri S, Sferrazza C, Rini GB, Di Fede G. Low serum levels of 25-hydroxy vitamin D in adults affected by thalassaemia major or intermedia. *Bone.* **2006;** 38: 888-92. http://dx.doi.org/10.1016/j.bone.2005.11.018

[18] Ha SY, Mok AS, Chu WC, Raskalkar DD, Cheuk DK, Chiang AK, Ho MH, Chan GC. A practical chelation protocol based on stratification of thalassemic patients by serum ferritin and magnetic resonance imaging cardiac T2*. *Hemoglobin.* **2009;** 33: 323-31. http://dx.doi.org/10.3109/03630260903211979

[19] Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, El-Hajj Fuleihan G, Josse RG, Lips P, Morales-Torres J; IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. Global vitamin D status and determinants

of hypovitaminosis D. *Osteoporos Int.* **2009;** 20: 1807-20.
http://dx.doi.org/10.1007/s00198-009-0954-6

[20] Hanley DA, Cranney A, Jones G, Whiting SJ, Leslie WD, Cole DE, Atkinson SA, Josse RG, Feldman S, Kline GA, Rosen C; Guidelines Committee of the Scientific Advisory Council of Osteoporosis Canada. Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. *CMAJ.* **2010;** 182: E610-8.
http://dx.doi.org/10.1503/cmaj.080663