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ORIGINAL PAPER

Beta-thalassaemia major: Prevalence, risk factors and clinical consequences of hypercalciuria

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Summary

Regular transfusion and chelation therapy produces increased life expectancy in thalassaemic patients who may develop new complications. Since few data are available regarding hypercalciuria in β-thalassaemia major (TM), the aim of our study was to evaluate its prevalence, risk factors and clinical consequences. We enrolled 176 adult TM patients followed at the Center of Thalassemia of Ferrara. Hypercalciuria was defined by a calciuria of 4 mg/kg/day or more in a 24-h urine sample. Anamnestic, biochemical and radiological data were collected. Hypercalciuria prevalence was reported in 69.3% of patients (females 52.5%). Hypercalciuric (HC) patients used deferasirox (DFX) more often than normocalciuric (NC) patients (47.5% vs 29.6%; $p < 0.05$). In HC subjects plasma parathyroid hormone (PTH) (24.1 ± 10.4 vs 30.1 ± 13.2 pg/ml) and phosphate levels (3.6 ± 0.5 vs 3.8 ± 0.7 mg/dl) were lower, whereas serum calcium (9.6 ± 0.4 vs 9.4 ± 0.4 mg/dl) and urinary 24-h phosphaturia (0.9 ± 0.4 vs 0.6 ± 0.3 g/day) were higher as compared to NC patients ($p < 0.05$ for all comparisons). Supplementation with oral calcium and cholecalciferol was similar between the groups. A higher rate of kidney stones was present in HC (14.8%) versus NC patients (3.7%) ($p < 0.05$). Hypercalciuria is a frequent complication in adequately treated adult TM patients. Hypercalciuria prevalence is increased in DFX users whereas haemoglobin level or calcium supplements play no role. A significant proportion of HC patients developed kidney stones.

KEYWORDS
deferasirox, hypercalciuria, renal disease, thalassemia

INTRODUCTION

β-Thalassaemia syndrome is an inherited disorder due to defects in the synthesis of the β-haemoglobin chain characterized by chronic anaemia and ineffective erythropoiesis.[1–6] Blood transfusions cause iron overload and organ damage because of the production of reactive oxygen species.[2–5] Chelating therapy with deferoxamine (DFO),

Abbreviations: DFX, Deferasirox; HC, Hypercalciuric; NC, Normocalciuric; PTH, Parathyroid Hormone; TM, β-thalassaemia major.

deferiprone (DFP) or deferasirox (DFX) reduces the clinical consequences of iron overload.[1–6] Owing to the introduction of regular transfusions and the use of new iron chelators, the increase in overall survival of thalassaemic patients in the western world is associated with the development of new complications, including hypercalciuria.[1–6] Hypercalciuria is defined by daily urinary calcium excretion of 4 mg/kg/day or more, or 250 mg/day or more in women and 300 mg/day or more in men, and may be associated with the development of kidney stones and osteoporosis in the general population.[7,8] Chronic hypoxia due to anaemia, haemolysis and kidney iron overload may impair renal tubular function, causing hypercalciuria in non-adequately transfused/chelated thalassaemic patients.[9–17] In addition, thalassaemic patients treated with DFX frequently display renal tubular dysfunction.[18–30] Furthermore, high transfusion rates and important bone-marrow suppression are associated with decreased creatinine clearance and increased hypercalciuria rates and severity.[31] However, few data are, so far, available on hypercalciuria in adequately treated adults with β-thalassaemia major (TM). Therefore, the aim of our study was to evaluate prevalence, risk factors, and clinical complications of hypercalciuria in adult β-TM patients.

METHODS

This cross-sectional, retrospective study enrolled β-TM patients attending the Thalassemia Center of Ferrara, Italy. All patients had been receiving regular blood transfusions, maintaining pre-transfusion haemoglobin levels between 9 and 10.5 g/l according to international guidelines.[2] Data on transfusion regimen, ongoing therapies (including chelation therapy), laboratory/instrumental investigations and clinical complications were reviewed from medical records.

Exclusion criteria were: age less than 18 years, pregnancy and breastfeeding, reduced kidney function (estimated glomerular filtration rate below 60 ml/min/1.73 m^2), severe liver disease (cirrhosis with Child Pugh C; alanine aminotransferase exceeding five times the upper limit of normal), primary hyperparathyroidism, hypoparathyroidism, genetic renal tubular diseases and neoplastic hypercalcaemia.

The following biochemical data were considered in the analysis: 24-h urinary calcium, phosphate, creatinine and protein excretion, serum parathyroid hormone (PTH), calcium, 25-OH vitamin D, phosphate, uric acid, sodium, potassium, magnesium, zinc, creatinine, total protein, ferritin and soluble transferrin receptor. Hypercalciuria was defined by urinary calcium excretion of 4 mg/kg/day or more in a normal 24-h urine sample (defined by creatininuria: 20 mg/kg/day in men; 15 mg/kg/day in women). Biochemical exams were taken by standard methods.

Diagnosis of kidney stone disease was based on a history of urinary tract stones or obstruction as well as kidney and bladder ultrasound examination. Bone mineral density (BMD) was assessed by dual X-ray photon absorptiometry using Hologic Bone Densitometry (Hologic Horizon DXA scanner, Bedford, MA, USA) and was expressed as T-score and Z-score. Scans were performed on lumbar spine (L1–L4) and on total and neck femoral sites as previously described.[32] Participants at least 50 years old were diagnosed with osteoporosis in the case of a T-score of −2.5 or less or osteopenia if T-score was between −2.4 and −1. Patients younger than 50 years old were diagnosed with reduced bone mass if Z-score was −2 or less. Vertebral fractures were detected by X-ray morphometry according to the Genant criteria, carried out by a radiologist with expertise in differentiating vertebral fractures from vertebral deformities in thalassaemia patients.[33]

Liver stiffness measurement by transient elastography was evaluated by FibroScan (Echosens, Paris, France).[34] Magnetic resonance imaging was employed to evaluate cardiac and liver iron overload by measuring the T2* signal, and liver iron content (LIC), as previously described.[35] Endocrinopathies were diagnosed according to current guidelines.[2]

Statistical analysis was performed by means of Fisher's exact test for qualitative measures, by a *t*-test for parametrically distributed quantitative measures, and by the Mann–Whitney test for non-parametrically distributed quantitative measures. Values of $p < 0.05$ were considered significant. Multivariate regression models were developed to analyse the relationship between calciuria and other characteristics (PTH, phosphate, calcium, 25-OH vitamin D, uricaemia, ferritin and urinary 24-h phosphate), using calciuria as the dependent variable and adjusting for age and sex. Data in the tables and figures apply to the entire sample and there are no missing observations for any of the anamnestic data or laboratory tests. This study was approved by the Local Ethic Committee and informed consent was obtained from all patients (protocol number CE-AVEC 697/2020/Oss/AOUFe).

RESULTS

In all, 176 patients with TM (57.4% female) with a mean age of 44.9 ± 7.6 years old (21–64 years old) and a body mass index (BMI) of 23.1 ± 3.3 kg/m^2 were enrolled in the study. All patients were on regular transfusion and chelation therapy. Hypercalciuria was diagnosed in 69.3% of TM patients (122 subjects). Hydrochlorothiazide (HCT) treatment was recorded in 15 hypercalciuric (HC) patients (12.3%) and in none of the normocalciuric (NC) patients ($p < 0.05$). Mean age, BMI, age at first transfusion, transfusion frequency, age at start of chelation, splenectomy rate and age at splenectomy were similar in HC and NC patients. Sex distribution in HC and NC patients was significantly different, but rates for males and females were similar in HC patients (see Table 1).

Hypercalciuria prevalence was similar in all decades of age, indicating that this complication may also occur in young patients. In HC patients DFX was the most used chelation therapy (47.5%), followed by DFO (18%), DFP (13.1%) and combination therapy. The rate of HC patients treated with DFX was significantly higher as compared

TABLE 1 Characteristics of β-thalassaemia major (TM) patients

Patients: No	All 176	HC 122	NC 54	*p*
Females: *N* (%)	101 (57.4)	64 (52.5)	37 (68.5)	<0.05
Males: *N* (%)	75 (42.6)	58 (47.5)	17 (31.5)	
Age (years old):				
Mean ± SD (range)	44.9 ± 7.6 (21–64)	45.5 ± 7.1 (21–62)	44.8 ± 7.5 (25–64)	NS
Median (IQR)	46 (9)	46 (9)	43.5 (10)	
BMI (kg/m^2):				
Mean ± SD (range)	23.1 ± 3.3 (16.8–36.9)	22.9 ± 3.3 (16.8–36.9)	23.3 ± 6.2 (17.6–32.4)	NS
Median (IQR)	22.6 (3.9)	22.4 (3.6)	23.2 (3.8)	NS
Age at first transfusion (months):				
Mean ± SD (range)	14.9 ± 13.4 (2–60)	14.4 ± 11.9 (2–60)	16.38 ± 16.37 (2–60)	NS
Median (IQR)	10 (18)	10 (18)	8 (20)	
Transfusions interval (days):				
Mean ± SD (range)	17.2 ± 4.1 (7–31)	16.9 ± 4 (7–29)	17.9 ± 4.1	NS
Median (IQR)	17 (6)	16.5 (6)	17 (5)	
Age at start of chelation (years old):				
Mean ± SD (range)	6.7 ± 4.3 (1–16)	6.6 ± 3.2 (1–16)	7 ± 2.6 (3–13)	NS
Median (IQR)	6 (7)	6 (7)	6 (3.5)	
Splenectomy *N* (%)	114 (64.8)	82 (67.2)	32 (59.2)	NS
Age at splenectomy (years)				
Mean ± SD (range)	15.4 ± 9.4 (2–42)	15.1 ± 10.1 (2–38)	16.3 ± 11.3 (2–42)	NS
Median (IQR)	13.5 (15)	13.5 (14)	13 (13.8)	

Note: $p < 0.05$ was considered as statistically significant (HC vs NC patients).

Abbreviations: BMI, body mass index; HC, hypercalciuric patients; IQR, interquartile range; NC, normocalciuric patients; NS, not statistically significant; SD, standard deviation.

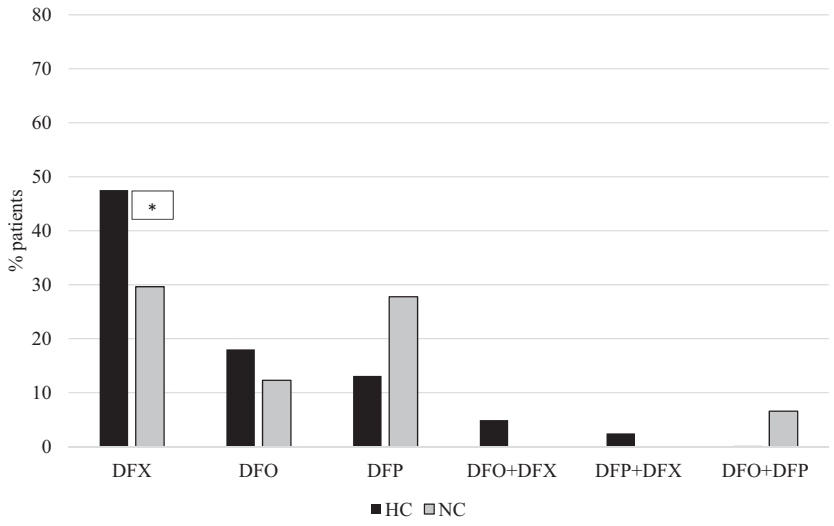

FIGURE 1 Percentage of patients using different types of chelation therapy and comparison between Beta thalassaemia major (TM) hypercalciuric and normocalciuric patients. DFX, deferasirox; DFO, deferoxamine; DFP, deferiprone; HC, hypercalciuric patients; NC, normocalciuric patients. *$p < 0.05$

to NC patients (47.5% vs 29.6%; $p < 0.05$) (Figure 1). Iron-chelating treatment dose was similar in HC and NC patients for all drugs, and DFX treatment duration was similar between HC and NC subjects (5.6 ± 4 vs 5.8 ± 3.4 years). Plasma ferritin and LIC were lower in HC than in NC patients, whereas cardiac and liver T2* were similar in both patient groups, regardless of the type of iron chelator used (Table 2).

Plasma PTH and phosphate levels were significantly higher in NC patients while calcium plasma levels, 24-h urinary phosphate, creatininuria and proteinuria levels were significantly higher in HC patients, regardless of the type of

TABLE 2 Iron overload parameters in hypercalciuric (HC) and normocalciuric (NC) patients

Mean ± SD median (range) median (IQR)	HC	NC	*p*
Ferritin (ng/ml)	667.2 ± 779.3 (54–7074)	935.1 ± 1167.3 (69–8200)	<0.05
	477 (518)	556 (928.8)	
STR (ng/ml)	3.4 ± 1.4 (0.94–7.55)	3.2 ± 1.3 (1.1–7.75)	NS
	3.15 (1.85)	2.95 (1.38)	
Heart T2* (ms)	35.5 ± 12.5 (2–48)	34.2 ± 15.8 (6–48)	NS
	38 (6)	37 (7)	
Liver T2* (ms)	12.5 ± 8.8 (0.5–34.3)	10.7 ± 8.9 (0.56–31.87)	NS
	10.2 (13.9)	7.4 (17.1)	
LIC (mg/g/dw)	4.3 ± 5.5 (0.94–51)	6.8 ± 8 (0.95–45.6)	<0.05
	2.8 (3.1)	3.6 (5.9)	
LSM (KPa)	5.8 ± 2.7 (2.8–14)	6.7 ± 4.3 (3.5–26.4)	NS
	5.5 (2.1)	5.6 (2.6)	

Abbreviations: IQR, interquartile range; LIC, liver iron concentrations; LSM, liver stiffness measurement; NS, not statistically significant; SD, standard deviation; STR, soluble transferrin receptor.

chelator used by participants (Table 3). Furthermore, 24-h urinary phosphate levels did not differ among HC patients according to the type of chelator (0.95 ± 0.51 g/day with DFX use and 0.87 ± 0.37 g/day with other chelators), while plasma uric acid and protein levels were significantly lower in HC patients under DFX treatment compared with HC subjects not taking DFX (3.6 ± 1.3 vs 4.5 ± 1.2 mg/dl and 7.2 ± 0.5 vs 7.6 ± 0.7 g/dl respectively, $p < 0.05$).

In the multivariate regression model, calciuria revealed a significantly negative correlation with plasma phosphate and uric-acid levels ($p < 0.01$), and a significantly positive correlation with plasma calcium and 24-h urinary phosphate levels ($p < 0.01$). No correlation was found with PTH, 25-OH vitamin D, 24-h urinary protein, ferritin, age and sex (Table 4).

Kidney complications

Symptoms related to kidney stones were reported in 15.6% of HC patients as compared to 3.7% of NC subjects ($p < 0.05$). On ultrasound examination, kidney stones were detected in 18 HC patients (14.8%) and in 2 NC patients (3.7%) ($p < 0.05$). Bilateral kidney stones, hydronephrosis and nephrocalcinosis were only found in HC patients (6.6%, 2.5% and 1.6% respectively).

Vitamin D supplementation and bone status

Cholecalciferol supplementation was administered at similar rates in HC and NC patients (73.8% vs 81.5%) and at similar doses (31 468.5 ± 18 749.3 iu/month; range 10 000–100 000 iu/month vs 31 824.3 ± 17 582.5 iu/month; range 10 000–56 000 iu/month respectively). In addition, calcifediol supplementation was administered to 2.5% of HC patients (2154 ± 1590 iu/month) and to none of the NC patients. One HC patient used alfa-calcidiol and none used calcitriol. Calcium carbonate was administered at similar rates in HC and NC patients (4.1% vs 9.2%) and at similar doses (533.3 ± 83.3 mg/day vs 700 ± 273 mg/day).

Out of 122 HC patients, 89 were less than 50 years old (72.9%) and 33 were 50 years old or older (27.1%), whereas out of 54 NC patients 39 were less than 50 years old (72.2%) and 15 were 50 years old or older (27.8%). In subjects less than 50 years old, a similarly low BMD was found in both HC and NC patients (71.9% vs 64.1%). In subjects 50 years old or older, osteoporosis was found to be similar in HC and NC patients (72.7% vs 86.7%); osteopenia was found in 27.3% of HC versus13.3% of NC patients. The rate of subjects treated with anti-osteoporotic therapy was slightly higher in HC patients (31.1%) as compared to NC patients (24.1%), but this difference did not reach statistical significance. A slightly higher rate of vertebral fractures was found among HC compared to NC patients [36.8% vs 31.5% respectively, not significant (NS)]. Similarly, hypogonadism rates overlapped in the two groups (67.2% in HC vs 72.2% in NC patients).

DISCUSSION

Our study is the first to evaluate prevalence, risk factors, and clinical complications of hypercalciuria in a population of adequately transfused and chelated adult TM patients. Few studies have previously investigated kidney dysfunction in patients with transfusion-dependent thalassaemia (TDT) (including sickle cell disease, thalassaemia intermedia and E/β-thalassaemia) and all of them exclusively enrolled patients undergoing DFX treatment or inadequately transfused.[18,25,31] Therefore, our study is the first to investigate hypercalciuria in a homogeneous group of well-compensated TM patients.

We found a higher hypercalciuria rate in TM patients (69.3%) as compared to that reported in the general population (0.6%–12%)[7,8] similar to that described by Capolongo

TABLE 3 Biochemical parameters in β-thalassaemia major (TM) hypercalciuric and normocalciuric patients

Mean ± SD (range) median (IQR)	HC	NC	*p*
Calciuria (mg/day)	435.1 ± 161.1 (245–1401)	142.8 ± 51.8 (31–234)	<0.01
	414 (163.3)	142.5 (69)	
PTH (pg/ml)	24.2 ± 10.4 (8–77)	30.1 ± 13.2 (10–78)	<0.05
	22 (11)	28.5 (17.3)	
Calcium (mg/dl)	9.6 ± 0.4 (8.7–10.5)	9.4 ± 0.4 (8.5–10.5)	<0.05
	9.65 (0.5)	9.5 (0.7)	
Phosphate (mg/dl)	3.6 ± 0.5 (2.1–4.8)	3.8 ± 0.7 (2.6–5.9)	<0.05
	3.6 (0.7)	3.8 (0.98)	
25(OH)vit D (ng/ml)	28.5 ± 9.6 (5.8–59.7)	27.4 ± 10.1 (7.2–54.3)	NS
	28.4 (13.9)	27.7 (9.9)	
Magnesium (mg/dl)	2.1 ± 0.2 (1.49–2.77)	2.1 ± 0.2 (1.7–2.4)	NS
	2.12 (0.2)	2.06 (0.3)	
Zinc (mg/dl)	89.41 ± 22.23 (49–131)	90.1 ± 32.28 (52–130)	NS
	88 (24.5)	88.5 (20.5)	
Potassium (mg/dl)	4.45 ± 0.38 (3.5–5.5)	4.47 ± 0.38 (3.6–5.4)	NS
	4.4 (0.5)	4.45 (0.5)	
Sodium (mg/dl)	139.03 ± 2.32 (129–145)	139.26 ± 2.33 (132–146)	NS
	139 (2)	139 (2)	
Creatinine (mg/dl)	0.70 ± 0.16 (0.31–1.14)	0.66 ± 0.21 (0.3–1.23)	NS
	0.7 (0.2)	0.7 (0.2)	
Uricaemia (mg/dl)	4.01 ± 1.33 (1.5–7.4)	4.42 ± 1.42 (1.7–7.6)	NS
	3.9 (1.9)	4.35 (1.9)	
24-h urinary phosphate (g/day)	0.9 ± 0.4 (0.3–4)	0.60 ± 0.3 (0.1–1.3)	<0.01
	0.9 (0.5)	0.6 (0.4)	
Proteinuria (mg/day)	213.17 ± 148.28 (37–1169)	188.3 ± 203.12 (28–1266)	<0.05
	171 (114.8)	117 (78)	
24-h urinary creatinine (g/day)	1.35 ± 0.5 (0.8–5)	1.16 ± 0.3 (0.5–1.9)	<0.05
	1.3 (0.4)	1.1 (0.3)	
Plasma proteins (g/dl)	7.36 ± 0.63 (5.9–10)	7.22 ± 0.51 (6.3–9.1)	NS
	7.2 (0.7)	7.25 (0.6)	
Pretransfusional Hb (g/l)	97 ± 5 (79–110)	96 ± 5 (85–108)	NS
	97 (5)	96 (7)	

Note: $p < 0.05$ was considered as statistically significant (HC vs NC patients).

Abbreviations: IQR, interquartile range; Hb, haemoglobin; HC, hypercalciuric patients; NC, normocalciuric patients; NS, not statistically significant; PTH, parathyroid hormone; SD, standard deviation.

et al. (60%).[25] Wong *et al*. reported higher HC, but the study included several TDT types,[18] whereas Quinn *et al*. reported lower rates (28.7%),[31] but they included participants less than 18 years old, not adequately transfused and with other TDT. However, when selecting only patients adequately transfused and chelated, hypercalciuria rate and severity were higher.[31] Taken together, these results would suggest that chelating therapy may play a role in HC development. We found that DFX treatment was significantly more frequent in HC as compared to NC patients, in line with the evidence that thalassaemic patients treated with DFX frequently display renal tubular dysfunction.[18–30,36,37] In addition, plasma ferritin and LIC levels were lower in HC as compared to NC patients, regardless of the type of chelation, supporting the role of chelation treatment in the pathogenesis of hypercalciuria. Several mechanisms have been suggested to explain this finding. Iron overload chelation may deplete cellular iron storage and remove enzymatic iron from pathways that control glomerular filtration,[26–31] and DFX may have direct cytotoxic effects on proximal renal tubules similar to the Fanconi syndrome.[26–31] In keeping with the hypothesis of tubular damage,[19] in our series, 24-h urinary phosphaturia, creatininuria and proteinuria were higher in HC as compared to NC patients, and there was a significantly positive correlation between calciuria and 24-h urinary phosphate levels. Lower uric-acid levels in HC patients under DFX

TABLE 4 Relationship between 24-h urinary calcium and biochemical parameters. Multivariate regression analysis was performed using calciuria as the dependent variable; PTH, calcium uricemia, phosphate, 25-OH vitamin D, 24-h urinary phosphate and ferritin as independent variables; data are adjusted for age and gender. Regression coefficients (coef.) and *p*-values are shown

	24-h urinary calcium	
	Coefficient	p
PTH (pg/ml)	−0.51	NS
Calcium (mg/dl)	104.56	<0.01
Phosphate (mg/dl)	−78.93	<0.01
Uricaemia (mg/dl)	−32.19	<0.01
24-h urinary phosphate (g/day):	267.49	<0.01
25-OH vitamin D (ng/ml)	1.97	NS
24-h urinary protein (mg/day):	0.013	NS
Ferritin (ng/ml)	0.009	NS
Age (years old)	0.28	NS
Gender[a]	−45.17	NS

Abbreviations: NS, not statistically significant; PTH, parathyroid hormone.
[a]Gender: 1 females and 0 males. $p < 0.05$ was considered as statistically significant (HC vs NC patients).

treatment as well as the negative correlation between calciuria and plasma uric-acid levels may account for increased urate urinary excretion due to tubulopathy, even though we did not analyse 24-h urinary uric-acid values. Moreover, in our study, the duration and dose of DFX treatment were similar in HC and NC patients unlike those described by Wong *et al.*[18]

A minority of HC patients were treated with HCT, which was unable to control hypercalciuria. These findings are in line with the evidence that, in the general population, HCT is ineffective in controlling hypercalciuria in patients displaying hyperphosphaturia.[38] In fact, we found higher urinary phosphate excretion in HC as compared to NC patients, which could possibly explain HCT failure.

In the general population, hypercalciuria is more common in females than males,[7,8] while, in our group, we found that males and females were equally distributed among HC patients, suggesting that gender is not a risk factor for hypercalciuria in TM patients.

Our study is the first to show that hypercalciuria rates are similar in the different decades of life, suggesting that this complication should be monitored from a young age.

We found that PTH levels were significantly lower in HC as compared to NC patients, whereas HC subjects displayed higher plasma calcium levels as well as higher 24-h urinary phosphate levels compared to NC patients, suggesting that PTH actions are exerted by a different hormone.

It has recently been demonstrated that non-thalassaemic hypercalciuric patients display high plasma levels of fibroblast growth factor 23 (FGF23).[38–40] Intact FGF23 (iFGF23) is secreted by osteocytes and osteoblasts and has a phosphaturic action, possibly causing kidney stones.[40] Therefore, FGF23 could mediate alterations in phosphate handling by the kidney and thus play a role in hypercalciuria in thalassaemic subjects. The increased iFGF23 levels in TM may be due to a reduced degradation of the intact product[41–43] or to a stimulatory effect of erythropoietin on iFGF23 production.[44] On the other hand, Stefanopoulus *et al.*[42] found reduced plasma Klotho levels in TM patients as compared to a control group, and Baldan *et al.*[45] showed low Klotho levels in hypercalciuric TM patients. Since Klotho is highly expressed in the kidney and activates the FGF23 signal pathway, these data suggest that kidney damage in TM may prevent Klotho production, impairing FGF23 action on the kidney with a consequent limited phosphaturic action.[42]

In our study, iron overload and anaemia did not seem to be associated with renal damage, as has been reported previously.[18–25] Indeed, previous studies enrolled inadequately chelated thalassaemic patients, indicating that pathogenetic mechanisms may differ according to treatment status.[19–22] Splenectomy and younger age of splenectomy were found to be equally frequent in both HC and NC patients, suggesting that these conditions do not associate with hypercalciuria development in our patient sample. Our findings differ both from those of Ricchi *et al.*[46] who described that splenectomy was associated with nephrolithiasis in non-TDT patients, and those of Demosthenous *et al.*[19] who showed that splenectomy was a risk factor for the development of tubulopathies.

In our study, kidney stones were significantly more frequent in HC as compared to NC patients. This finding is in line with the literature, describing that kidney stones are more likely to develop in HC patients and in DFX users.[47–49] Kidney stones are associated with reduced BMD in the general population, whereas in thalassaemia this association has not been fully demonstrated.[42,50–52] A retrospective study on TDT patients identified an association between urolithiasis, reduced femoral BMD, and an increased risk of fracture in males.[23] On the other hand, our study found a similar distribution of bone mineral deficits in HC as compared to NC, suggesting that hypercalciuria may not impact the compromised bone health in TM patients. However, this finding might be influenced by treatment with the antiresorptive drugs of these patients.

In the case of hypercalciuria, Thalassaemia International Federation (TIF) guidelines state that it is necessary to maintain good hydration and avoid excessive intake of animal protein and salt, whereas food calcium intake should not be avoided.[2,42] In persistent cases, or when a significant bone disease is present, thiazide diuretics (TZD) are recommended at the lowest possible doses because of the risk of hypotension.[2,42] In our series, a minority of HC patients was treated with HCT because of the frequent occurrence of hypotension, and the drug was unable to control hypercalciuria. These findings are in line with the evidence that, in the general population, HCT is ineffective in controlling hypercalciuria in patients displaying hyperphosphaturia.[53] We did, in fact, find higher urinary phosphate excretion in HC as compared to NC patients, which may account for HCT failure. TIF guidelines also suggest reducing the doses of oral calcium or calcitriol supplements

in the case of hypercalciuria, maintaining 25-OH vitamin D within the low normal range. In our series, 25-OH vitamin D was within the normal range and did not differ between HC and NC patients. In addition, the frequency and dose of vitamin D administered was similar between the two groups, as was the calcium supplement, suggesting that vitamin D levels and calcium treatment are not a risk factor for hypercalciuria and do not impact the development of kidney complications. However, in our practice, we maintain vitamin D values at the lower limit of the normal range, preferring cholecalciferol to other vitamin D formulations (i.e. calcitriol and calcifediol).

Strengths of the study

Our study is the first to evaluate differences between HC and NC adequately transfused and chelated adult TM patients, and furthermore to investigate the risk factors and consequences of hypercalciuria. The strength of the study is the high number of subjects investigated, all of whom were adequately transfused and chelated and all adult, with a mean age higher than that of patients reported in previous studies.

Limits

A limitation of this study is represented by the retrospective design. We did not measure iFGF23 and cFGF23 levels, which are not included in routine clinical practice. In addition, the four commercially available immunoassays for their measurement differ substantially since they use different antibodies targeting different epitopes on the FGF23 protein. Moreover, they use different units of measure and different calibration, and lack harmonization.[54] To enhance our findings, future research should investigate FGF23 and 1,25 (OH)2 vitamin D plasma levels in HC thalassaemic subjects. In addition, we had neither data on 24-h urinary sodium, potassium and uric-acid levels, nor urinary β-2-microglobulin levels (marker of tubular damage), so we did not evaluate other features of tubular dysfunction. Finally, DEXA results may be influenced by the concomitant use of anti-resorptive drugs.

CONCLUSIONS

Hypercalciuria is a frequent complication in adequately treated adult TM patients and pathophysiological mechanisms may differ according to the use of adequate transfusion/chelation regimens. DFX may cause hypercalciuria, most likely through tubular damage. Anaemia and oral supplementation with calcium/cholecalciferol do not seem to be associated with hypercalciuria. HC TM patients should be monitored for the development of renal damage and osteoporosis.

AUTHOR CONTRIBUTIONS

Ludovica Aliberti and Irene Gagliardi performed the research, analysed and interpreted the data and wrote the paper. Andrea Ziggiotto wrote the paper and performed the research. Aldo Carnevale performed the research. Maria Rita Gamberini designed the study and interpreted the data. Martina Verrienti, Maria Chiara Zatelli and Marta Bondanelli critically revised the manuscript. Maria Rosaria Ambrosio designed the study, performed the research and interpreted the data and made a critical revision of the manuscript. All the authors gave final approval for this version to be published and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CONFLICT OF INTEREST

Ludovica Aliberti, Irene Gagliardi, Maria Rita Gamberini, Andrea Ziggiotto, Aldo Carnevale, Martina Verrienti, Marta Bondanelli, Maria Chiara Zatelli and Maria Rosaria Ambrosio have no conflicts of interest to report.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available on request from the corresponding author.

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