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Introduction

Thalassaemia is a disorder of haemoglobin synthesis due to mutations in the globin chains (± or ²). In its more severe form, it requires treatment with chronic transfusion and concomitant iron chelation therapy to prevent iron overload. The long-term complications include cardiac, liver, hormonal and metabolic bone disease but the nature of renal dysfunction in thalassaemia is poorly characterized.[1]

In a retrospective analysis, we reported symptomatic urolithiasis to be present in 18.1% of patients with transfusion-dependent thalassaemia,[2] compared with 5.2% in the general population.[3] Urolithiasis is associated with osteoporosis in the general population,[4] and we have previously identified an association between symptomatic urolithiasis and reduced bone mineral density (BMD) in patients with ² -thalassaemia major.[2]

Establishing the composition of urolithiasis by biochemical analysis is challenging as collection of stones requires careful compliance by patients, biasing data collection. Dual-energy computer tomography (DECT) enables the composition of renal tract stones to be determined *in situ* and non-invasively by analysing how substances behave at two different photon energies.[5]

The prevalence and composition of asymptomatic urolithiasis, and its risk factors, in ² -thalassaemia major remain unknown. Using DECT, serum and urine biochemical analysis and dual energy X-ray absorptiometry (DXA), we prospectively established the prevalence, composition and risk factors for urolithiasis in an asymptomatic cohort of patients with ² -thalassaemia major.

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Methods

Subjects

Monash Health hosts the state centre for management of patients with thalassaemia in Victoria, Australia. Adult patients with ² -thalassaemia major, who did not have symptoms of urolithiasis, were enrolled consecutively and gave written informed consent. Clinical data pertaining to transfusion, iron chelation and osteoporosis therapy were documented. Previous bisphosphonate use and fractures were documented based on medical records and radiology findings as appropriate.

Bone mineral density

DXA was assessed at the lumbar spine (L2-L4), femoral neck and total body using a GE Lunar Prodigy (Madison, Wisconsin, software version 12).

Biochemical assessments

Biochemical measurements were obtained concurrently with, or within 1 month of, the DXA study. These comprised serum creatinine, 25(OH) vitamin D, calcium, phosphate, parathyroid hormone, uric acid, bicarbonate, ferritin and haemoglobin. A concurrent morning urine sample was obtained for pH, calcium, protein, uric acid and creatinine. Hypercalciuria was defined as a urine calcium to creatinine ratio (UCa/Cr) greater than 0.40 mol/mol.[6] The UCa/Cr has been found to be strongly correlated with 24 hour urinary calcium excretion.[7] Increased urine uric acid and protein tubulopathy was defined as a urine uric acid to creatinine ratio >0.8 and urine protein to creatinine ratio >0.3, respectively.[8]

Dual energy computed tomography (DECT)

Non-iodinated contrast renal tract CT was performed using the Discovery CT750 HD system with Gemstone Spectral Imaging (GSI) (GE Healthcare, Milwaukee, USA). All subjects underwent an initial single energy enhanced spiral CT of the renal tracts. If renal tract calculus/calculi were detected, the size and location were confirmed, and simultaneous acquisitions of dual-energy data using 140kVp and 80 kVp energy levels targeted on the calculi were undertaken. The composition of the renal tract calculi was then derived from a standardized atomic number plot.

Statistical analysis

The association between urolithiasis status and potential explanatory variables was determined using the Mann Whitney U test. Correlations between UCa/Cr, deferasirox dosage and BMD parameters were determined using the Spearman's rank test. Analyses were conducted using SPSS 20 (IBM, Armonk, NY).

Results

Twenty-seven subjects were enrolled with a median age of 41 with 59% being male (Table 1). Deferasirox was introduced in 2007 with all subjects having previously received desferioxamine. In the deferasirox group the mean duration of drug exposure was 6.52±1.6 years. Of the 2 subjects who were taking desferoxamine, the duration of treatment was 5 years (2009-) and 4 years (2010-); deferasirox was previously taken for 2 years (2008-2009) and 3 years (2007-2010) in those 2 subjects respectively.

The prevalence of urolithiasis was 59% (16/27) with equal prevalence amongst males and females. There were 51 stones in 16 affected subjects ranging between 1 mm and 13 mm in diameter. In subjects with kidney stones, 69% (11/16) had multiple stones and 56% (9/16) had stones of variable composition. The most common stone types were struvite (33%), calcium oxalate (31%) and cystine (22%) (Table 2).

Subjects with urolithiasis had higher BMI (24.4 mg/kg^2 vs 22.2 mg/kg^2, P=0.04). There was no difference in urine parameters, serum calcium, 25(OH) vitamin D, ferritin, creatinine or weight-adjusted deferasirox dosage and urolithiasis status. Interestingly, the UCa/Cr and urine tubular protein levels were elevated in 21/27 (78%) and 7/27 (26%) of subjects, respectively (with no statistical difference between groups). Stone formers had significantly lower serum magnesium levels.

There was no significant difference in BMD between subjects with or without stones. In a subgroup analysis, subjects with calcium-containing stones had lower femoral neck Z-scores (-2.40 vs -0.2, P=0.04) but this was not significant at the lumbar spine. Moreover, patients with calcium stones had higher serum creatinine (76 µmol/L vs 65 µmol/L, P=0.02) and lower ferritin

levels (432 µg/L vs 1017 µg/L, P=0.02). There was a significant positive correlation between UCa/Cr with deferasirox weight-adjusted dosage (R=0.537, P=0.008) in the overall group.

Discussion

This study non-invasively identified the prevalence and composition of asymptomatic urolithiasis in patients with ²-thalassaemia major. We report a high burden of urolithiasis (multiple and variable composition) and hypercalciuria. Calcium containing stones are associated with reduced femoral neck BMD, highlighting the association between renal disease and bone disease in transfusion-dependent thalassaemia.

The ability of DECT to differentiate between distinct stone categories has been demonstrated in both phantom models[5] and *in-vivo* studies.[9] Other studies have also validated the accuracy of DECT in distinguishing between stones composed of uric acid, cysteine, struvite, calcium oxalate and calcium phosphate.[5, 10] These different stone entities reflect the heterogeneity of stone types seen in this study.

Calcium stones were present in 37% of subjects and are also the most common stone type in the general community.[11] We did not find calcium or vitamin D supplementation to be a risk factor for kidney stones, as is the case in the general population.[12] Hypercalciuria has previously been reported in 28.7% of thalassaemia subjects on deferoxamine.[13] In this study, hypercalciuria is present in 78% of our patient cohort treated with deferasirox, and in a prospective study, we reported deferasirox to be associated with dose-dependent hypercalciuria.[14] We did not find a significant association between hypercalciuria and urolithiasis; however, hypomagnesemia was associated with urolithiasis, and an association between hypomagnesemia and hypercalciuria has been reported.[15]

In the general population, hypercalciuric stone formers have reduced BMD.[16] We found a significant association between calcium-containing stones and reduced femoral neck Z-scores in our study. The lack of a significant association at the lumbar spine or total body Z-score may

reflect the difficulty in assessing BMD at sites of marrow expansion, reduced truncal height and bony deformity in thalassemia major.[2]

In a 19-year longitudinal study of transfusion-dependent thalassaemia, accelerated loss of bone mass coincided with a change in iron chelator therapy from deferoxamine to deferasirox.[17] Whether the relationship between bone loss and deferasirox is causal remains to be defined; but reports of dose-dependent hypercalciuria[14] and renal phosphate wasting from Fanconi syndrome[18] are possible mechanisms for bone loss. Moreover, the findings of an increased serum creatinine and reduced ferritin concentrations in calcium stone formers in this study may implicate deferasirox in the renal-bone axis in thalassaemia.[1]

The particularly high prevalence of struvite and cysteine stones in this cohort is in contrast to the general population. Struvite stones (calcium, phosphate and magnesium) are often associated with urinary tract infections and high urine pH.[11] Reports of reduced complement levels, neutrophil dysfunction, increased risk of infection post-splenectomy[19] and stone obstruction may predispose to urine infections in thalassaemia. The high incidence of struvite stones may suggest undetected chronic or recurrent urinary infection in our subjects. Cysteine stones make up 5% of all stones and are mainly caused by an inherited defect in renal tubular transport.[11] The high prevalence of cysteine stones in our study may indicate renal disease or treatment-induced renal tubulopathy given the high proportion of subjects with tubular proteinuria and hypercalciuria. Uric acid stones represented only 2% of all stones in this study. The small numbers of uric acid stones may reflect regular blood transfusion (3-4 weekly basis), which may limit the degree of erythroid turnover, and hence serum uric acid levels. Our subjects also had alkaline urine pH levels which would also tend to reduce the risk of uric acid stone formation.

We are mindful of the limitations of this study. The small sample size may overestimate the prevalence of kidney stones in this group. Screening for more extensive renal tubulopathy using

renal tubular phosphate, amino acid and ^{2}2-microglobulin wasting was not performed. Although, we did not have a control group, the prevalence of asymptomatic urolithiasis was 2% in a prospective study of 715 healthy subjects who underwent abdominal ultrasound screening.[20]

Conclusions

Urolithiasis is highly prevalent and of mixed composition in the thalassaemia. Stones composed of calcium are associated with reduced femoral neck BMD. Where available, DECT may be a sensitive, safe and non-invasive strategy for screening patients with urolithiasis and determining stone composition. Careful monitoring for renal tubulopathy (in particular hypercalciuria) and osteoporosis is indicated in patients with thalassaemia major.

Author contributions: PW had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: PW, SRP, KKL

Acquisition of data: PW, SRP, KKL, DJ, DHO, JCGD

Analysis and interpretation of data: PW, SRP, KKL, FM, PJF, PGK, JCGD, MTG, DKB

Drafting of the manuscript: PW, SRP, KKL, FM, PJF, PGK, JCGD, DHO, DJ, MTG, DKB

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Abstract

Asymptomatic urolithiasis is common and of mixed composition in patients with ²-thalassaemia major. Twenty-seven subjects were imaged using dual-energy computer tomography to determine the presence and composition of urolithiasis. The prevalence of urolithiasis was 59% and affected patients generally had multiple stones, often with more than one component: struvite (33%), calcium oxalate (31%) and cystine (22%). Hypercalciuria was present in 78% of subjects and calcium-containing urolithiasis was associated with reduced femoral neck Z scores.

Urolithiasis is prevalent and are associated with reduced bone mineral density in ² -thalassaemia major

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