The association of bone mineral density and parathyroid hormone with serum magnesium in adult patients with sickle-cell anaemia

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Abstract

Introduction: Bone disorders including osteopenia and osteoporosis are a frequent cause of morbidity in sickle-cell disease (SCD). Magnesium (Mg) regulates some biological processes important in bone remodelling. We aimed to investigate whether serum Mg levels (sMg) may have an impact on bone mineral density (BMD) in sickle-cell anaemia (SCA).
Material and methods: Sixty adults with SCA in steady-state and 20 age- and race-matched healthy blood donors were included in the study. The BMD was evaluated with respect to minerals and biochemical indices of bone metabolism. Multivariate analysis was performed to determine the factors influencing BMD.
Results: The mean sMg concentration was 0.64 ±0.06 (reference range 0.7-1.2 mmol/l) for 34% of the population, and 0.86 ±0.08 mmol/l for 66%. There were significant differences between Mg groups and controls in BMD, phosphorus (PO_4), parathyroid hormone (PTH) ($p = 0.011$, $p = 0.011$ and $p = 0.0001$ respectively) and osteocalcin (OC) ($p = 0.030$) levels. The sMg was found to be associated positively with serum calcium (Ca), PTH and OC ($r = 0.585$; $r = 0.436$; $r = 0.351$ respectively, all at $p < 0.05$), and negatively with PO_4 ($r = –0.312$; $p < 0.05$). Multivariate analysis demonstrated that only PTH ($p < 0.05$) was an independent factor for BMD. Moreover, it identified sMg, OC, and CTX as independent factors for PTH (all $p < 0.05$).
Conclusions: These results indicate that serum Mg may be a co-contributing factor in causing low BMD. However, other possible aetiologies including decreased PTH and increased bone turnover certainly play a role. Based on the present data, it is prudent to monitor sMg routinely in this patient population and treat the condition whenever possible.

Key words: sickle-cell anaemia, magnesium, calcium, bone mineral density, parathyroid hormone, osteoporosis, bone turnover.

Introduction

Sickle-cell disease (SCD) is an autosomal recessive genetic disease with an increase in the adhesion of sickled erythrocytes, and it is a potential cause of vaso-occlusive episodes, which are related to clinical manifestations, morbidity and mortality [1, 2]. The SCA often coincides with osteoporosis or osteopenia in children and young adults [3]. Accumulated evidence suggests that tissue injury and adverse effects on bone are usually produced by increased erythropoiesis and hypoxic conditions secondary

to the obstruction of blood vessels by sickled erythrocytes [4, 5].

Magnesium (Mg) plays an important role in the pathophysiology of erythrocyte sickling in SCA [6]. Magnesium has also been shown to slow clotting time [7, 8], which might reduce vascular blockage and improve blood flow in SCD. Clinical studies showed that Mg deficiency, which is partially due to increased urinary Mg excretion [9], induces dehydration and morphological abnormalities in erythrocytes, promotes vasoconstriction and enhances vascular endothelial injury, leading to hypoxia in multiple organs including the osseous tissue [10]. Additionally, Mg regulates biological processes important in bone remodelling such as calcium absorption, parathyroid hormone (PTH) secretion, osteoblast adhesion and bone formation [11-13]. The Mg deficiency produces hormonal and skeletal disturbances that have been associated with a number of bone disorders including osteoporosis [14, 15]. This has been supported by experimental magnesium deficiency studies showing that insufficient dietary Mg intake increased osteoclast number, and bone loss in laboratory animals [16].

This does not appear to have been studied in patients with sickle-cell anaemia and this prompted us to assess the prevalence of Mg deficiency in a group of adult patients with SCD, and to analyse the relationships between bone turnover markers with serum Mg to determine the role of Mg in the pathogenesis of bone changes seen in SCD.

Material and methods

Subjects

Adult patients with steady state SCD within the age range 20-40 years visiting the outpatient clinic of the university hospital were asked to participate in the study. Twenty age and race matched HbAA healthy blood donors were enrolled in the study as the control group. Ethical approval was obtained from the research and scientific committee of the university. Informed consent was obtained from all participants after explanation of the nature of the study. History was taken and clinical examination was done and followed by appropriate investigations to rule out any secondary disorders that might be causing osteoporosis. Patients who were on steroids, had anorexia nervosa, hyperthyroidism, chronic obstructive pulmonary disease, liver disease, inflammatory bowel disease, or had deranged renal functions (serum creatinine > 2.5 mg/dl) were excluded from the study.

Bone mineral density measurements

Bone mineral density (BMD) and T-score analyses were done at lumbar spine L2, lumbar spine L4 and total body BMD twice by dual-energy X-ray absorptiometry (DXA) imaging (Hologic QDR 2000, Bedford, MA). T-scores are standard deviations of patient BMD compared with BMD of young, healthy, sex-matched adult controls. T-score values were calculated based on data of young adults aged 20-40 years [17]. Cases studied were classified as osteoporosis when T-score was ≤ 2.5 and osteopenia when T-score was between –1.0 and –2.5 at one or more anatomical sites, consistent with WHO criteria [18].

Biochemical tests

All blood samples were collected at 10 am after overnight fasting. Serum samples were separated by centrifugation at 3000 rpm for 10 min, and then were stored in the freezer at –80°C until analysis. Serum calcium (Ca), magnesium (Mg) and phosphate (PO_4) were measured using an endpoint assay in a Dade Behring Dimension RxL clinical chemistry system (Dade Behring; Germany) using standard procedures [19]. Total calcium concentration was adjusted for serum albumin. The laboratory reference ranges are 2.2-2.6 mmol/l for Ca, 0.8-1.2 mmol/l for Mg, and 1.0-1.4 mmol/l for PO_4.

Intact serum parathyroid hormone (iPTH) levels were measured by electrochemiluminescence immunoassay (Roche Diagnostics, Germany) [20]. The reference range for iPTH was 15-65 pg/ml (1.6-6.9 pmol/l), with analytical sensitivity of < 0.1 pmol/l and intra- and inter-assay CV (coefficient of variation) values less than 5% and 7%, respectively. Serum b-ALP was detected using an EIA kit (OSTASE® Bone Specific Alkaline Phosphatase EIA, Immunodiagnostic Systems Inc, Fountain Hills, AZ, USA) [21]. Analytical sensitivity for the b-ALP assay was < 1 U/l (reference range between 50 U/l and 136 U/l) with an intra- and inter-assay variability lower than 10.1% and 10%, respectively. Serum intact OC level was assessed by a solid-phase enzyme-amplified sensitivity immunoassay kit (hOST-EASIA; BioSource Europe S.A., Nivelles, Belgium). This immunoassay is specific for measurement of intact form of osteocalcin with proven absence of cross reactivity with other osteocalcin fragments [22]. Analytical sensitivity was < 0.4 ng/ml (reference range between 6.8 ng/ml and 32.2 ng/ml) with intra- and inter-assay CV values of 5.2% and 6.7% respectively. The serum marker of bone resorption C-terminal telopeptide of type-I collagen (CTx) was measured by a two-site ELISA (Serum Crosslaps one-step; Osteometer Biotech, A/S, Denmark). The reference range is between 0.13 ng/ml and 4.1 ng/ml and intra- and inter-assay CV of the assay is 5.2% and 6.7%. Serum N-terminal telopeptide of type-I collagen (NTx), a specific biochemical indicator of bone resorption, was measured using an enzyme immunoassay (Osteomark® Wampole Laboratories Inc., Princeton, NJ, USA) with detec-

Table I. Characteristics and laboratory data of healthy controls and SCD patients

Parameter	SCD Group (n = 60)	Control group (n = 20)	Value of p
Age [years]	22.37 ±7.40	22.87 ±4.91	0.631
Mg [mmol/l]	0.74 ±0.14	0.85 ±0.17	0.044
Ca [mmol/l]	2.11 ±0.13	2.36 ±0.19	0.022
PO_4 [mmol/l]	1.32 ±0.25	1.14 ±0.23	0.006
PTH [pmol/l]	2.22 ±1.26	4.11 ±1.46	0.0001
b-ALP [U/l]	77.13 ±14.81	59.69 ±12.57	0.021
OC [ng/ml]	56.46 ±5.3	37.88 ±6.53	0.001
CTX [ng/ml]	0.41 ±0.16	0.36 ±0.16	0.017
NTX [ng/ml]	1.06 ±0.41	0.97 ±0.36	0.383

Mean ± SD, Mg – magnesium, Ca – calcium, PO_4 – phosphate, b-ALP – bone-specific alkaline phosphatase, OC – total osteocalcin, CTX-I – C-terminal telopeptide of type-I collagen, NTX – N-terminal telopeptide of type-I collagen

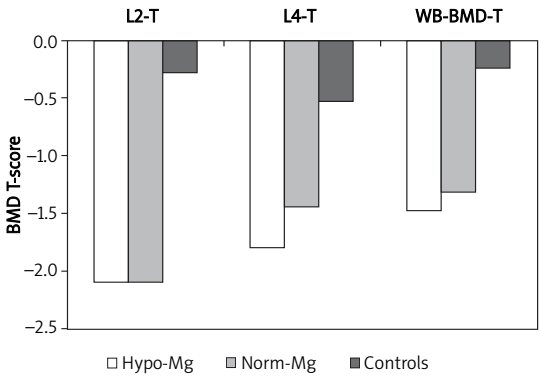

Figure 1. Bone mineral density T-score according to magnesium levels. ANOVA test revealed significant variation between groups at L2, L4 and WB-BMD ($p < 0.001$, $p < 0.05$ and $p < 0.001$ respectively)

tion limit of 2.5 nM bone collagen equivalent (BCE) (reference range: 10 nM to 60 nM BCE), and CV equal to 4.6% [22].

Statistical analysis

Results were expressed as mean ± standard deviation. χ^2 tests, ANOVA, and non-parametric tests for trend were used, as appropriate, to compare the proportion of patients with specified clinical characteristics. When significant differences were observed in ANOVA, Bonferroni post-hoc testing was done to identify differences between groups. Correlation analysis was done using linear regression and Pearson's correlation coefficient. Statistical significance was defined by a $p < 0.05$. All statistical tests were two-tailed. A sample size analysis demonstrated that 19 subjects would be required in each group to detect a statistically significant ($p < 0.05$) difference of 0.05 in serum magnesium level between groups (SD, 0.05; power, 88%). Calculations were done using SPSS for Windows (release 13.0, 2004; SPSS Inc., Chicago, IL).

Results

Sixty SCA patients, confirmed by previous haemoglobin electrophoresis and/or HPLC, were included in the study. The mean age of patients was 24.64 (±4.26) years (34 females and 26 males). The control group included 20 age-matched (26.73 ±5.84 years old) subjects (22 females and 18 males). The χ^2 analysis showed no significant differences between patients and control groups regarding age ($\chi^2 = 0.33$, $p > 0.05$) or sex ($\chi^2 = 0.64$, $p > 0.05$).

According to WHO criteria using T scores, 43 patients (71%) were osteoporotic or osteopenic at least in one of the three studied anatomical locations. The SCA patients showed significantly lower BMD T-score at L2, L4 and WB-BMD in comparison with controls ($p = 0.002$; $p = 0.035$; and $p = 0.008$ respectively). There was a significant decrease of serum Ca ($p = 0.022$) and serum PTH ($p < 0.0001$), and a significant increase in serum PO_4 ($p = 0.006$) in the patient group compared to the control group. Significant increases in b-ALP, OC, and CTX levels were also found in SCA patients compared to controls ($p = 0.021$, $p = 0.001$, $p = 0.017$ respectively); however, serum NTX demonstrated no significant change between groups (Table I).

To determine the impact of Mg deficiency in this group of SCA patients on other minerals, hormonal, and bone turnover markers, patients were divided according to their serum Mg concentrations into hypomagnesaemic (Mg < 0.7 mmol/l) and normomagnesaemic (Mg ≥ 0.7 mmol/l), and these parameters were compared to those of controls. Twenty patients (33.3%) had serum Mg levels lower than the normal range (0.7-1.2 mmol/l). These hypo-Mg patients had significantly lower BMD and T-score at all evaluated sites (L2, L4, and WB) than norm-Mg patients and controls (all $p < 0.05$) (Figure 1). Hypo-Mg patients showed lower Ca ($p < 0.001$) and PTH ($p < 0.001$) concentrations compared with norm-Mg patients and controls. Analyses of biochemical bone marker data showed that hypo-Mg SCA patients had significantly higher PO_4, b-ALP and OC concentrations in comparison to norm-Mg and controls ($p < 0.05$; $p < 0.05$ respectively). Serum CTx, and NTx concentrations were not significantly different between studied groups by ANOVA analysis; however, CTX levels were significantly higher in the hypo-Mg than the norm-Mg group according to the post-hoc Bonferroni test ($p < 0.05$).

Table III presents the univariate Pearson correlations between the studied parameters. Serum Mg showed significant positive correlations with Ca ($r = 0.736$, $p < 0.000$), PTH ($r = 0.446$, $p < 0.05$), and OC ($r = 0.358$, $p < 0.05$), and a significant negative

Table II. Comparison of studied parameters between controls and sickle-cell patients classified according to magnesium status

Parameter	SCD patients		Control group (n = 20)	ANOVA	
	Hypo-Mg[a] (n = 20)	Norm-Mg[b] (n = 40)		F	Sig.
Age [years]	23.40 ±3.58	21.40 ±6.09	22.87 ±4.91	1.156	0.506
Mg [mmol/l]	0.64 ±0.06[c,d]	0.86 ±0.08	0.85 ±0.17	17.669	0.0001
Ca [mmol/l]	2.04 ±0.34[c,d]	2.33 ±0.25	2.36 ±0.19	1.716	0.197
PO_4 [mmol/l]	1.23 ±0.32	1.28 ±0.27	1.14 ±0.23	4.963	0.011
PTH [ng/l]	2.22 ±0.95	2.20 ±1.34	4.11 ±1.46	8.604	0.001
b-ALP [U/l]	66.82 ±11.41	81.10 ±13.91	59.69 ±12.57	5.210	0.011
OC [ng/ml]	47.40 ±7.92	60.07 ±13.75	37.88 ±6.53	9.543	0.001
CTx [ng/ml]	0.36 ±0.07[c]	0.43 ±0.18	0.36 ±0.16	1.180	0.120
NTx (nM BCE)	0.92 ±0.43	1.15 ±0.38	0.97 ±0.36	1.069	0.355

[a]Hypo-Mg – serum Mg < 0.7 mmol/l, [b]Norm-Mg – serum Mg > 0.7 mmol/l, [c]p < 0.05 in comparison to controls, [d]p < 0.05 in comparison to hyper-Mg group

Table III. Univariate analyses of relationships between Mg and other markers of bone metabolism

Parameter	BMD		PTH		Ca		Mg	
	r[a]	Value of p	r	Value of p	r	Value of p	r	Value of p
BMD	1.000	–	0.329[b]	0.042	0.211	0.300	0.209	0.286
PTH	0.329[b]	0.042	1.000	–	0.231	0.247	0.446[b]	0.022
Ca	0.211	0.300	0.231	0.247	1.000	–	0.736[b]	0.0001
Mg	0.209	0.286	0.446[b]	0.022	0.736[b]	0.000	1.000	–
PO_4	–0.397[b]	0.030	0.043	0.828	0.390[b]	0.032	–0.342[b]	–0.022
b-ALP	0.010	0.957	0.152	0.441	0.269	0.167	0.049	0.641
OC	–0.378[b]	0.044	–0.550[b]	0.002	0.060	0.761	0.358[b]	0.031
CTX	–0.289	0.122	–0.379[b]	0.037	–0.135	0.493	0.081	0.748
NTX	–0.097	0.610	0.083	0.674	0.268	0.168	0.103	0.631

[a]Pearson correlation coefficient, [b]Significant correlation (2-tailed)

correlation with serum PO_4 (r = –0.312, p < 0.05). The BMD showed a significant negative correlation with phosphorus and osteocalcin (r = –0.397, p < 0.05; r = –0.378, p < 0.05 respectively). The PTH correlated positively with BMD (r = 0.329, p < 0.05), and Mg (r = 0.446, p < 0.05) and negatively correlated with OC (r = 0.550, p < 0.001) and CTX (r = 0.343, p < 0.05). Calcium showed a significant positive correlation with Mg (r = 0.736, p < 0.001) and PO_4 (r = 0.390, p < 0.05).

Table IV summarizes the multivariate analysis of relationships between either BMD or PTH with other studied parameters. To elucidate the significant determinants of BMD, we performed a multiple regression analysis that used the serum Ca, PO_4, Mg, PTH, OC, b-ALP, CTX, and NTX as the potential explanatory variables. It was found that PTH was the only independent determinant of BMD (p = 0.039). Multiple linear regression analysis also showed Mg, OC and CTX as independent determinants of PTH (all p < 0.05).

Table IV. Multivariate analysis of the relationships between BMD or PTH and other studied parameters

Parameter	[a]Dependent: BMD		[b]Dependent: PTH	
	β Coefficient	Value of p	β Coefficient	Value of p
BMD	–	–	0.145	0.373
PTH	0.413[c]	0.039	–	–
Ca	–0.055	0.792	–0.062	0.784
Mg	0.161	0.457	0.397[c]	0.012
PO_4	–0.288	0.133	–0.123	0.407
OC	–0.123	0.601	0.403[c]	0.013
b-ALP	–0.026	0.895	0.013	0.94
CTX	–0.131	0.553	0.399[c]	0.013
NTX	–0.13	0.508	0.039	0.806
Age	0.079	0.662	–0.016	0.918

[a]Dependent variable: BMD, predictor in the model: PTH; [b]dependent variable: PTH, predictors in the model: osteocalcin, magnesium, CTX; [c]significant correlation

Discussion

In the present study, patients with SCD showed significantly lower BMD concentration at L2, L4 and WB-BMD (all $p < 0.05$) than the control group. Furthermore, 30% of the patients were found to be osteopenic and 50% osteoporotic based on T-scores of the patients according to WHO guidelines. These results are accompanied by a significant reduction in PTH and serum Ca concentrations and increased concentration of bone turnover parameters (OC, b-ALP and CTX). These results are in accordance with those of previous literature [1, 23], and suggest a relation between low BMD and increased bone turnover, as previously reported [24].

It has been suggested that Mg deficiency may have a role in the development of osteoporosis [25-27]. We therefore investigated whether alterations in sMg may have a role in the pathogenesis of bone changes seen in sickle-cell anaemia. In the present study, Mg levels were lower than the normal range (0.7-1.2 mmol/l) in 34% of SCA patients. These patients showed significantly lower BMD values and T-score than norm-Mg patients and controls. These findings suggest that Mg deficiency may have a role in the decreased BMD found in our group of patients with SCA.

We then studied the inter-relationships between sMg and bone turnover parameters OC, b-ALP, CTX and NTX. Osteocalcin and b-ALP are phenotypic markers for terminally differentiated osteoblasts and early-stage differentiated osteoblasts, respectively [28]. We found that b-ALP and OC are increased both in hypo- and norm-Mg patients compared with controls. These findings are in agreement with previous studies reporting accelerated bone turnover in SCA patients [29]. In addition, we found that CTX levels are increased significantly in hypomagnesaemic SCA patients compared to the control group, which reflects the increased bone resorption. Moreover, CTX was found to be negatively correlated with PTH by univariate correlation analysis, and an independent negative determinant of PTH by stepwise multiple regression analysis.

The PTH is very important for skeletal health. It is synthesized and secreted by the chief cells of the parathyroid glands and is the principal hormone responsible for calcium homeostasis [30]. We further found that the hypo-Mg group had significantly lower PTH levels than either norm-Mg or controls, and that serum Mg correlated positively with serum PTH. These patients showed significantly reduced BMD. In addition, multivariate analysis identified PTH as the only predictive factor for BMD. These data suggest that a blunted PTH response in hypo-Mg patients may be the causative factor for BMD reduction in SCD. These results are supported by previous studies which suggested that Mg deficiency causes impairment of PTH release from the parathyroid and the refractoriness of bone and kidney to the hormone, which may lead to the suppression of bone remodelling [14, 25, 31, 32].

The reciprocal relationship between Mg and PTH is well documented [14]. Hypomagnesaemia appears to blunt the hypocalcaemic release of PTH [31], through the intermediary action of cAMP [33]. Magnesium is also required for the sensitivity of the target tissues to PTH and vitamin D metabolites [34, 35]. On the other hand, PTH has profound effects on Mg reabsorption in the kidney and gut and release from bone [36].

Another effect of PTH on the kidney is to stimulate loss of phosphate ions in urine by reducing the reabsorption of phosphate from the proximal tubule [37]. In the present study we found that PTH correlates negatively with PO_4 ($r = –0.321$, $p < 0.031$). Therefore, the decrease in PTH may explain the elevated serum PO_4 observed in these patients. It has been reported that modest increases in serum PO_4 levels significantly affect red cell metabolism, increase 2,3 diphosphoglycerate (DPG) levels, and cause decreased affinity of oxygen for haemoglobin. Such changes could lead to increased intravascular sickling and blood flow abnormalities [38], which have been associated with osteoporosis [23]. Moreover, low levels of total magnesium in sickle-cell erythrocytes have been linked to increased sickling due to cell dehydration [39]. In the present study, PO_4 was found to be negatively correlated with serum magnesium ($r = –0.342$, $p < 0.022$). These data suggest that increased serum PO_4 along with decreased serum Mg could represent additional pathogenic mechanisms contributing, in these cases, to low BMD.

In conclusion, we identified a group of SCD patients with reduced serum Mg levels, who had significantly lower BMD than their counterparts with normal Mg concentrations, suggesting that serum Mg may play an important role in bone metabolism through its association with PTH, OC and PO_4, and may provide additional information for the pathogenesis of bone loss in this disease. Finally, the present data warrant further investigation to determine whether serum Ca/PO_4 and PTH values normalize in SCA patients with Mg repletion in a prospective way, which will be an important and cost-effective intervention in this group of patients.

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