CLINICAL SCIENCE

Low bone mass density is associated with hemolysis in brazilian patients with sickle cell disease

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OBJECTIVES: To determine whether kidney disease and hemolysis are associated with bone mass density in a population of adult Brazilian patients with sickle cell disease.

INTRODUCTION: Bone involvement is a frequent clinical manifestation of sickle cell disease, and it has multiple causes; however, there are few consistent clinical associations between bone involvement and sickle cell disease.

METHODS: Patients over 20 years of age with sickle cell disease who were regularly followed at the Hematology and Hemotherapy Center of Campinas, Brazil, were sorted into three groups, including those with normal bone mass density, those with osteopenia, and those with osteoporosis, according to the World Health Organization criteria. The clinical data of the patients were compared using statistical analyses.

RESULTS: In total, 65 patients were included in this study: 12 (18.5%) with normal bone mass density, 37 (57%) with osteopenia and 16 (24.5%) with osteoporosis. Overall, 53 patients (81.5%) had bone mass densities below normal standards. Osteopenia and osteoporosis patients had increased lactate dehydrogenase levels and reticulocyte counts compared to patients with normal bone mass density ($p<0.05$). Osteoporosis patients also had decreased hemoglobin levels ($p<0.05$). Hemolysis was significantly increased in patients with osteoporosis compared with patients with osteopenia, as indicated by increased lactate dehydrogenase levels and reticulocyte counts as well as decreased hemoglobin levels. Osteoporosis patients were older, with lower glomerular filtration rates than patients with osteopenia. There was no significant difference between the groups with regard to gender, body mass index, serum creatinine levels, estimated creatinine clearance, or microalbuminuria.

CONCLUSION: A high prevalence of reduced bone mass density that was associated with hemolysis was found in this population, as indicated by the high lactate dehydrogenase levels, increased reticulocyte counts and low hemoglobin levels.

KEYWORDS: Osteoporosis; Sickle Cell Disease; Hemolysis; Bone Mass Density; Kidney.

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INTRODUCTION

Sickle Cell Disease (SCD) is an inherited disorder caused by a point mutation in codon 6 of the β-globin gene. This mutant form of hemoglobin (HbS) is capable of polymerization and complex molecular and structural changes within the erythrocyte. Hemolytic anemia and vaso-occlusion crises are the main features of sickle cell disease, and they occur in homozygotes (HbSS) or in compound heterozygotes, such as those carrying HbS and β-thalassemia ($HbS\beta^+$ or $HbS\beta^0$) or HbS and hemoglobin C (HbSC). The illness tends to develop gradually toward multisystem organ failure.[1]

Bone involvement is a frequent clinical manifestation of sickle cell disease that occurs during the acute phase and during painful vaso-occlusive crises. Bone involvement is a source of chronic, progressive disability, such as the long-term effects of sickle cell disease upon bone mass density (BMD), growth, and chronic bone damage in the form of avascular necrosis and osteomyelitis. Osteopenia and osteoporosis are often asymptomatic; however, pain, fractures, deformities, and vertebral collapse may occur and require chronic analgesia, mechanical support, and surgical interventions.[2-6] Several studies have shown an overall reduction of BMD in children with SCD.[7-9] The causes of BMD abnormalities in patients with SCD are probably

multifactorial. Several factors, including reduced hemoglobin levels, abnormally low body mass index (BMI), increased ferritin levels, vitamin D deficiencies and low plasma zinc or sex steroid levels, have been previously correlated with reduced BMD in adult patients with SCD.[3,10-13] There are a limited number of studies and conclusions regarding the risk factors for osteoporosis in adult SCD patients. The present study aimed to establish the prevalence of reduced BMD in adult patients with SCD and examine whether any correlation exists between reduced BMD and clinical characteristics.

SUBJECTS AND METHODS

The inclusion criteria for this study consisted of patients over 20 years of age with a precise clinical and laboratory diagnosis of SCD and regular follow-ups at the Hematology and Hemotherapy Center of the University of Campinas (Hemocentro-Unicamp).

All SCD patients who attended this center from March 2007 to June 2008 had their charts reviewed. To be included, a patient's chart had to contain all of the following data: hemoglobin (Hb) electrophoresis, gender, age, BMI (BMI = weight (kg)/height^2(m^2)), serum creatinine, estimated creatinine clearance, glomerular filtration rate (GFR), microalbuminuria, hemoglobin (Hb) levels, reticulocyte counts, serum lactate dehydrogenase (LDH) levels, and a BMD assessment. Microalbuminuria was measured using the nephelometric method with nocturnal 12-hour urine.[15] Creatinine clearance was estimated by the Cockcroft-Gault formula.[16] Glomerular filtration rate was estimated by measuring 51Cr-EDTA clearance.[17] The diagnosis of SCD was estimated by a pH 8.6 cellulose acetate electrophoresis, a solubility test, and HbA2 and HbF quantification.[14] All patients who are treated at the Hematology and Hemotherapy Center routinely underwent the aforementioned exams, which facilitated this retrospective study.

BMD was measured at the femoral neck and lumbar spine using a Lunar DPX® densitometer. We classified the patients according to the World Health Organization[18] using the T-score, which represents the number of standard deviations (SD) from the mean of normal subjects. Values below -2.5 at one or both sites were defined as osteoporosis. Values below -1 and above -2.5 were defined as osteopenia, and values above -1 at both sites were considered normal.[18]

The study cohort included 65 patients (40 females and 25 males) with a median age of 32.5 years (20-30 years old (y.o.), n = 17; 31-40 y.o., n = 25; 41-50 y.o., n = 17; and 51-64 y.o., n = 6). Patients who were receiving hydroxyurea were also included in the study. Patients with HbSC and HbSβ^+ genotypes were excluded from the study. The National Ethical Committee Board approved the study, and all patients provided written informed consent.

Statistical analyses

Clinical characteristics were compared between patients with normal BMD and those with low BMD (including both osteopenia and osteoporosis), normal BMD and osteopenia alone, normal BMD and osteoporosis alone and between osteopenia and osteoporosis. Statistical analyses were performed by comparing each independent variable between two groups using the Mann–Whitney test for numerical values and Fisher's Exact Test for age. A p-value of 0.05 or less was considered statistically significant.

RESULTS

Based on the BMD, patients were divided into three groups, including those with normal BMD, those with osteopenia, and those with osteoporosis. Osteopenia and osteoporosis patients were classified as having reduced, or low BMD. According to WHO criteria, 12 patients (18.5%; 4M/8F) had normal BMD, 37 patients (57%; 13M/24F) had osteopenia, 16 patients (24.5%; 8M/8F) had osteoporosis, and 53 patients had low BMD (81.5%, 21M/32F) at either L1-L4 or the femoral neck site. Among the 60 HbSS patients, 10 (16.5%) had normal BMD, 34 (57%) had osteopenia and 16 (26.5%) had osteoporosis. Among the 5 HbSβ^0 patients, 2 had normal BMD and 3 had osteopenia. The clinical characteristics that were significantly associated with SCD patients who had normal BMD, osteopenia and osteoporosis are shown in Table 1.

A comparison of individuals with reduced BMD values and those with normal BMD values revealed increased LDH (median = 535 U/L *vs.* 883.5 U/L; $p<0.0149$) and an elevated reticulocyte fraction (median = 8.6% vs. 11.75%; $p<0.0124$) in the individuals with reduced BMD. LDH levels were also significantly higher in individuals with osteopenia compared with those with normal BMD values. Patients with osteoporosis presented with increased LDH and reticulocyte counts and decreased Hb compared with individuals with normal BMD values ($p<0.05$). Hemolysis was statistically increased in patients with osteoporosis compared with those with osteopenia, as indicated by the

Table 1 - Clinical characteristics of patients with SCD who have normal BMD, osteopenia or osteoporosis. Data are presented as minimum-maximum (median). All p-values were obtained using the Mann Whitney test for numerical values and Fisher's Exact Test for age.

	Normal n = 12 (18.5%)	Osteopenia n = 37 (57%)	Osteoporosis n = 16 (24.5%)	(p) NL vs. Osteopenia	(p) NL vs. Osteoporosis	(p) Osteopenia vs. Osteoporosis
Male/Female	4/8	13/24	8/8	1.0	0.45	0.37
Age (years)	25-49 (32.5)	20-64 (35)	26-64 (41.5)	0.41	0.0165	0.0095
BMI (kg/m^2)	14.42-27.39 (18.45)	15.94-29.90 (20.65)	16.73-26.63 (19.36)	0.113	0.422	0.17
GFR (EDTA) (mL/min/1.73m^2)	83.0-219.0 (115.5)	48.0-224.0 (125.05)	39.4-132.0 (102.0)	0.69	0.20	0.02
LDH (U/L)	384-1141 (535)	316-2040 (846)	506-2605 (1123.5)	0.0469	0.0005	0.0037
Hb (g/dL)	6.8-10.5 (8.55)	5.5-12.5 (8.5)	4.6-9.9 (7.8)	0.35	0.0113	0.042
Retc (%)	4.26-12.54 (8.6)	3.67-33.62 (10.62)	9.5-34.44 (15.55)	0.149	<0.0001	0.0008
Retc ($\times 10^9$/L)	142.5-394.1 (216.95)	112.0-759.5 (231.1)	219.8-617.5 (359.25)	0.58	0.0018	0.0055

Abbreviations: BMI: bone mass index; GFR: glomerular fibration rate; LDH: lactate dehydrogenase; Hb: hemoglobin; Retc: reticulocyte.

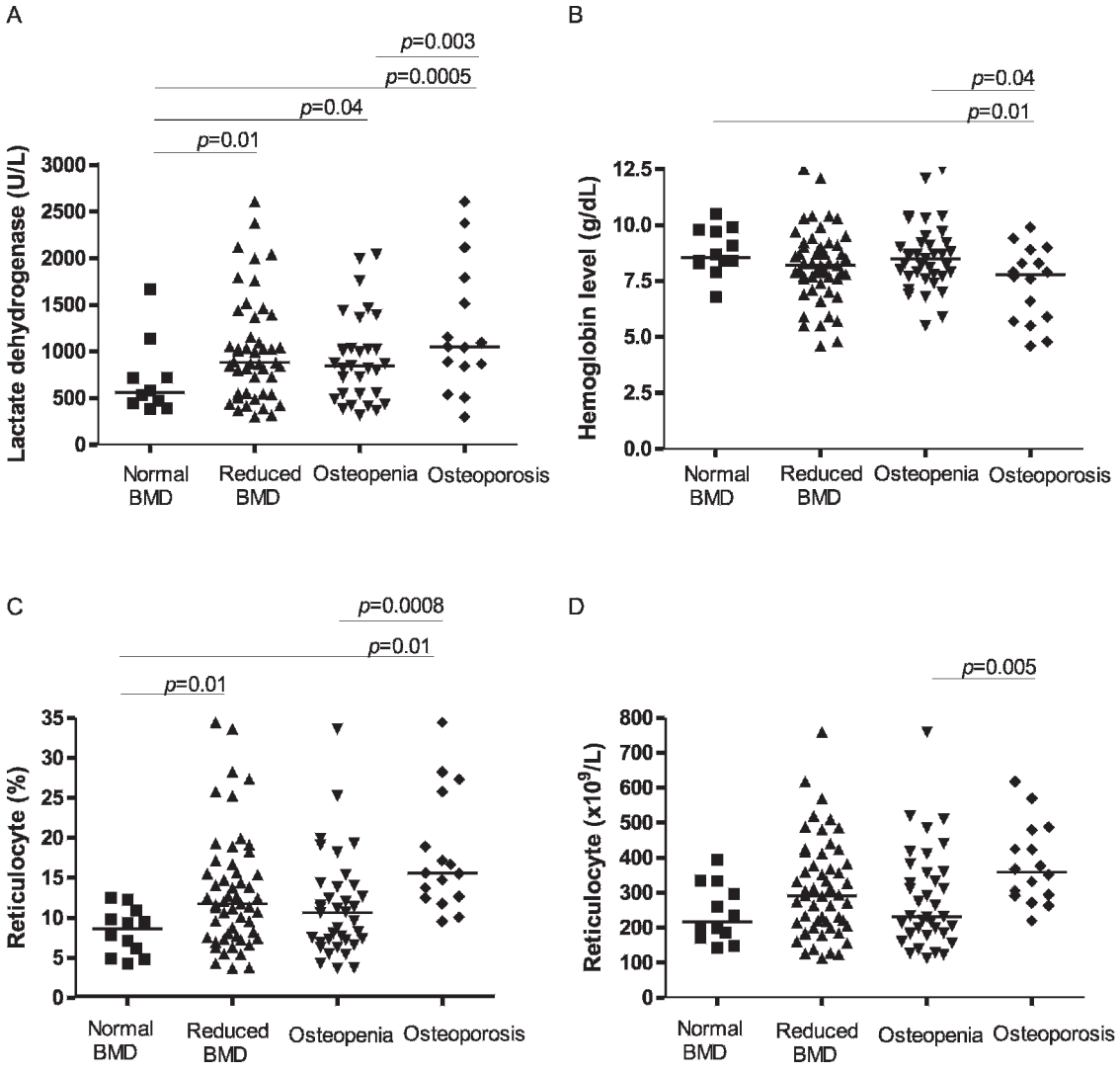

Figure 1 - (A) Lactate dehydrogenase levels (LDH); (B) Hemoglobin (Hb) values; (C) Percentage of reticulocytes and (D) Absolute reticulocyte counts in patients with normal bone mass density (BMD), reduced BMD (both osteopenia and osteoporosis), osteopenia alone and osteoporosis alone, as indicated in the figure. The horizontal bars indicate the median. The *p*-values are indicated in the figure.

increased LDH and reticulocyte counts (both by absolute values and percentage) and decreased Hb (Figure 1). The osteoporosis patient group was older and had a lower GFR than the osteopenia group. There was no significant difference between the groups with regard to gender, BMI, serum creatinine, estimated creatinine clearance, and microalbuminuria.

DISCUSSION

Our study indicated a high prevalence (81.5%) of low BMD in adults with HbSS or HbSβ^0 and revealed an association between low BMD and high LDH, high reticulocyte counts, and low hemoglobin levels in this population. The group of patients with osteoporosis also displayed a higher age and a lower GFR than patients with osteopenia. The life expectancy of SCD patients has improved due to the implementation of comprehensive sickle cell care. Concomitant with this increase in life expectancy, there is a desire to emphasize long-term health maintenance in these patients. Osteoporosis may be one of the major public health problems in SCD patients, particularly if the onset takes place at an early age.

Osteopenia and osteoporosis are well-known complications associated with SCD and thalassemia major; however, the information in the literature regarding the pathophysiology of bone diseases in adults with SCD is very limited.[3,6,10] Bones may be affected by both hemolytic and vaso-occlusive processes in SCD.[2] In our study, we found a correlation between low BMD and increased erythropoietic activity, which was assessed by the strong correlation between LDH, reticulocytes and Hb levels.

Serum lactate dehydrogenase has long been considered a useful clinical marker of intravascular hemolysis. Serum levels of lactate dehydrogenase are mildly elevated in illnesses involving extravascular hemolysis, such as immune hemolytic anemia; however, lactate dehydrogenase levels are substantially elevated in conditions associated with intravascular hemolysis, such as thrombotic thrombocytopenic purpura and paroxysmal nocturnal hemoglobinuria.

Although two thirds of all hemolysis occurs extravascularly in SCD, the remaining one third of red cells undergoes intravascular hemolysis.[19] Rapid scavenging of nitric oxide (NO)[16] by cell-free hemoglobin and oxygen free radicals, together with low concentrations of the substrate L-arginine,[20,21] reduces NO bioavailability in SCD. NO plays a role as a cytoprotective mediator, inhibiting the gene transcription of pro-adhesive and pro-inflammatory molecules, such as endothelial VCAM-1 and P-selectin.[22] Therefore, we speculated that reduced NO bioavailability could be related to low BMD in SCD. Moreover, in our study, older adults with SCD were found to have a higher prevalence of low BMD, which could be related to the chronic inflammatory state of SCD. The pathophysiology of low BMD in patients with chronic inflammation has been suggested to be related, in part, to increased bone resorption that results from the action of inflammatory cytokines, such as TNF-alpha and IL-6.[23] Inflammatory cytokines are similarly elevated in patients with SCD[24,25] and may play a role in the pathophysiology of low BMD.

Chronic and severe anemia places a burden on the bone marrow, with increased erythropoiesis causing hyperplasia of the bone marrow, a decrease in the trabecular network and osteopenia[7] and subsequent bone destruction.[6] Some studies, however, found no correlation between Hb levels and BMD values.[7,10] Corroborating our findings, Sarrai et al.[3] also described an association between abnormal BMD and reduced Hb levels. These conflicting data could be a result of subject selection. Both previous studies included patients with SS, SC, $S\beta^+$ and $S\beta^0$ hemoglobinopathies. Our study did not include SC and $S\beta^+$ patients, who typically present with high Hb levels, decreased hemolysis and normal GFR values that might obscure the impact of hemolysis and GFR on BMD.

Patients with osteoporosis also presented with mild renal impairment when compared to those with osteopenia. Renal impairment might be implicated in the pathogenesis of bone loss in these patients because increased bone resorption and osteoporosis are known to occur in patients with renal dysfunction.[26] However, hyperparathyroidism was not evaluated in these patients, and further studies would be necessary to better elucidate the mechanism by which renal impairment affects bone loss in SCD patients. Interestingly, Miller et. al[10] found no correlation between parathyroid hormone concentrations and osteoporosis in SCD patients.

Other clinical characteristics that are known to be risk factors for osteoporosis, such as the age of menopause in female patients, the amount of physical exercise, serum calcium values, other nutritional facts, or family history, were not evaluated in this study. Additional studies are necessary to reveal the independent impact of each of these characteristics on bone involvement.

CONCLUSION

A high prevalence of reduced BMD was found in Brazilian patients with SCD, and this loss of BMD was associated with hemolysis, as indicated by high LDH levels, elevated reticulocyte counts and low Hb levels. Although osteoporosis does not usually cause noticeable symptoms in patients who are accustomed to living with pain, osteoporosis may nevertheless lead to severe complications. There is no specific preventative therapy for this population, and, as a result, SCD health care should focus on calcium intake, vitamin D supplementation and reductions in hemolysis. Future research should evaluate the ability of hydroxyurea or pharmacological therapies to prevent bone mass loss in patients with SCD and low BMD.

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