Predictors of Abnormal Bone Mass Density in Adult Patients with Homozygous Sickle-Cell Disease

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ABSTRACT

BACKGROUND: Adult patients with sickle-cell disease (SCD) often have multiple bone complications causing tissue hypoxia and osteonecrosis. The impact on bone abnormalities lesion detected by bone mass density is not well-defined.

AIM: The study is a cross-sectional, perspective was designed to assess the prevalence of abnormal BMD in adult Bahraini patients with SCD and to assess the predictive risk of different metabolic variables such as serum level of vitamin D3, testosterone, and parathyroid hormone in addition to lactate dehydrogenase (LDH), hemoglobin (Hb), and reticulocyte count for the development of abnormal bone density on dual X-ray absorptiometry (DXA) scan.

METHOD: The study was conducted over the period of 12 months from first of January 2012 to end of December 2012. All patients were evaluated clinically for severity of SCD and abnormal bone mass density (BMD) using DXA scan. Blood samples were withdrawn for measuring the serum level of vitamin D3, testosterone, and parathyroid hormone in addition to Hb, LDH, and reticulocyte count. Multiple logistic regression analysis was used to assess risk prediction of different variables for the development of abnormal BMD on DXA with T-score ≤−2.5 standard deviation (SD).

RESULTS: The study included Bahraini patients with SCD (n = 55, age 29.24 ± 9.47 years, male 60% and female 40%) compared with an age-matched healthy control group (n = 55, age 28.82 ± 8.64 years, with 62% male and 38% female). Of the 55 patients with SCD compared with the control group, there were 33 (59%) patients with low BMD and 2 (3%) in the control. Among the 33 patients with SCD and with low BMD, there were 20 (36%) with osteoporosis (T-score of ≤−2.5 SD) and 13 (24%) with osteopenia (T-score of −1 to −2.5 SD). The most affected site of low BMD was lumbar spine (55%), followed by the radius (30%) and neck of the femur (15%). SCD patients with osteoporosis compared with the healthy subjects had significantly lower body surface area (BSA, m²) of 1.4 ± 0.3 vs. 1.63 ± 0.5 BMI, low level of vitamin D3 of 21.11 ± 6.95 ng/mL vs. 46.2 ± 15.19 (P < 0.001), lower testosterone level of 1.34 ± 0.54 vs. 2.18 ± 0.56 ng/mL (P < 0.001), higher reticulocyte count (P < 0.001), and higher LDH level (P < 0.001). The low serum level of vitamin D3 (<20 ng/mL) and low testosterone of <0.9 ng/mL had risk prediction (odds ratio) of 1.14 and 1.2, respectively, for abnormal BMD in SCD. In the risk prediction of other variables of parathormone (PTH), LDH, and reticulocyte, were not significant.

CONCLUSION: The prevalence of abnormal bone mass density (BMD) is high (60%) in Bahraini patients with SCD. There is significant low serum level of vitamin D3 and low testosterone hormone in those with very low bone mass density (BMD) (osteoporosis and T-score ≤−2.5). The low serum level of vitamin D3 (<20 ng/mL) and low testosterone of <0.9 ng/mL had risk prediction (odds ratio) of 1.14 and 1.2, respectively, for abnormal BMD in SCD.

KEYWORDS: sickle-cell anemia, bone mass density, vitamin D, Bahrain, osteoporosis, osteopenia

Introduction

Sickle-cell disease (SCD) is a hereditary hemolytic anemia characterized by the synthesis of an abnormal hemoglobin (HbSS). The incidence of SCD in the Arabian Peninsula is in the range of 1.2–2.6%. The loss of red blood cell (RBC) elasticity is the main pathophysiology of SCD. The normal RBC is elastic, allowing the cell to deform to pass through capillaries. In SCD, the low oxygen tension in blood promotes RBC sickling, causing repeated episodes of sickling that damage the cell membrane and decrease the cell elasticity. These cells fail to return to its normal shape when normal oxygen tension restored. As a consequence, the rigid blood cells are unable to deform as they pass through the narrow capillaries, leading to vessel occlusion and ischemia. The actual anemia of any illness is caused by hemolysis and destruction of RBC inside the spleen because of misshape as cells survive 10–20 days only.

Patients with SCD often develop vaso-occlusive crises and recurrent episodes of hemolytic anemia, causing hypoperfusion infarction and multiorgan dysfunction. SCD complications can be of acute nature with acute chest syndrome, pulmonary arterial hypertension, renal insufficiency, and cerebral vascular accident. Bone involvement in patients with SCD varies from acute clinical manifestations of painful vaso-occlusive syndrome or osteomyelitis to more chronic and debilitating complications, such as osteonecrosis, osteoporosis, osteopenia, narrow capillaries, leading to vessel occlusion and ischemia.
impaired growth, and chronic infections. Osteopenia and osteoporosis are often asymptomatic but may cause bone pain, fractures, bone deformity, and vertebral collapse, requiring chronic analgesia and mechanical support or surgical interventions. Chronic hemolysis leads to bone marrow hyperplasia and subsequent bone deformity. Although these bone complications may not contribute directly to increased mortality; they are a major source of comorbidity and adverse effect on patients’ quality of life.

There have been many advances in the understanding of the pathophysiology of vaso-occlusive disease, such as the interaction of red cells with endothelial cells. The reaction with adhesion molecules, cytokines, and polymorphonuclear neutrophils further clarified the understanding of the SCD clinical manifestations.

Despite these advances, the effect of SCD on bone mass density remains poorly elucidated, and many investigators agree that SCD patients are more likely to be osteopenic and osteoporotic. The clinical manifestations of SCD patient vary depending on the methods of evaluation and study populations, ethnicity, and geographical distribution. Many patients may have traditional risks of osteoporosis, including low body weight status, hypogonadism, and vitamin D deficiency.

The low BMD in children with SCD is well documented, but the status of bone mass density in adult patients (age >18 years) who may live until the fifth decade is not clear.

The present study aims to (1) validate dual X-ray absorptiometry (DXA) in the assessment of bone mass density in patients with SCD, (2) establish the prevalence of abnormal bone mass density in adult patients with SCD in Bahrain, and (3) determine the predictive risk of different variables for the development of abnormal BMD scan such as serum level of testosterone, vitamin D3, and ferritin.

Material and Methods

Study population. A total of 55 adult Bahraini patients aged >18 years and above with history of homozygous SCD were enrolled in the study.

The study was conducted over 12 months, from January 2012 to December 2012. Patient selection was consecutive from those patients attending the hematology clinic in Salmaniya Medical Complex.

These patients were compared with 55 age-matched patients who serve as a control group; those patients were referred to orthopedic clinic for nonspecific bone pain that turned to be normal on skeletal X-ray. Control patients have no history of SCD and their hemoglobin (Hb) on electrophoresis is HbAA, and they agree to be enrolled in the study.

Inclusion criteria. Each patient with diagnosis of homozygous SCD was included, adult age was defined as age of >18 years. All patients signed an informed consent. The study protocol was approved by the Research Committee of the Salmaniya Medical Complex, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Exclusion criteria. Patients with SCD were excluded if they had history of acute chest pain in the prior three weeks, hemoglobinopathy other than SCD, bone fracture, severe renal failure, or hepatic failure.

Material and clinical data. Patients’ height and weight were checked, and body surface area (BSA) was calculated for each patient. Each patient gave a full medical history about the frequency of chest pain, bone pain, and previous hospital admissions and current medications, including hydroxyurea.

Blood sample was withdrawn for the serum level of Hb, reticulocyte count, lactate dehydrogenase (LDH) enzyme, ferritin, vitamin D3 (cholecalciferol), testosterone, and parathyroid hormone.

Patients were classified into three groups based on clinical severity of SCD:

(a) Low severity: zero hospital admission for 12 months and no use of hydroxyurea.
(b) Medium severity: with one or two hospital admissions but no use of hydroxyurea.
(c) High severity: with more than three hospital admissions and use of hydroxyurea.

Hydroxyurea medication is used in painful crises in order to reduce pain frequency; the use of hydroxyurea was included as a marker of high clinical severity.

DXA was performed for every patient in the study as per study protocol. The World Health Organization (WHO) defined osteopenia as a BMD value between −2.5 and −1.5 standard deviation (SD) below the mean T-score at one or more bone sites and osteoporosis as <-2.5 SD below this norm. DXA reports from three different anatomical sites, i.e. femoral neck, lumbar spine (L1–L4), and ultradistal region of the radius were used to determine the BMD. The term low BMD was defined as a T-score between −1.5 and −2.5 SD below the norm and very low BMD was defined as a score of <-2.5 SD below the norm. The DXA machine used for assessment of all patients was GE Healthcare Lunar Prodigy Advance with V9.15 software.

Osteoporosis was defined as a T-score ≤ −2.5 on BMD and osteopenia as a T-score < −1 to −2.5 at one or more anatomical sites, which is consistent with WHO criteria. T-scores are the SD of BMD of patients compared with BMD of healthy gender-matched adult control.

Patients were divided into SCD with normal BMD and those with abnormal BMD; the patients with abnormal BMD were then subdivided as those with low and very low bone mass density.

Statistical analysis. Statistical Package for the Social Sciences (SPSS) version 20 was used for data entry and analysis. Clinical characteristics and biometric data of patients with SCD and the normal population were presented as mean ± SD. T-test
Table 1. Clinical characteristics and biometric data of patients with SCD and the normal population (data presented as mean ± SD).

| Variable                  | Control (n = 55) | SCD (n = 55) | P-value |
|---------------------------|-----------------|--------------|---------|
| Age (years)               | 28.82 ± 8.64    | 29.24 ± 9.47 | 0.809   |
| Male                      | 34 (62%)        | 33 (60%)     | 0.845   |
| Female                    | 21 (38%)        | 22 (40%)     |         |
| BSA                       | 1.63 ± 0.5 m²   | 1.42 ± 0.3 m² | 0.021  |
| Ferritin (n: 20–300 ng/L) | 80.09 ± 9.02    | 317.35 ± 105.80 | <0.001 |
| Haemoglobin (g/dL)        | 9.985 ± 0.71    | 8.731 ± 0.79  | <0.001  |
| PTH (n: 10–65 pg/mL)      | 41.56 ± 6.43    | 68.96 ± 7.73  | <0.05   |
| Testosterone (n: 0.2–4.3 ng/ml) | 2.18 ± 0.56  | 1.34 ± 0.54  | <0.001  |
| Vitamin D3 (n: >50 ng/mL) | 46.20 ± 15.19  | 21.11 ± 6.95  | <0.001  |
| Reticulocyte count (<2.5%)| 2.08 ± 0.42     | 6.12 ± 0.94   | <0.001  |
| LDH (n: 208–460 U/L)      | 195.95 ± 23.56  | 485.36 ± 142.28 | <0.001 |

Abbreviations: PTH, parathyroid hormone; BSA, body surface area; LDH, lactate dehydrogenase.

was used to compare whether the mean difference between two groups is statistically significant. Patients were divided into low BMD, very low BMD, and normal BMD groups.

Table 3 shows Analysis of variance (ANOVA) testing the mean difference between the three groups, with post hoc analysis.

Multiple logistic regression analysis was performed to assess the predictive value of various variables in the development of abnormal bone mass density in SCD patients; the variables were female gender, vitamin D3, testosterone hormone, parathormone (PTH), LDH levels, and reticulocyte count. All reported P-values were two tailed, and P-value was regarded as significant at the level of <0.05.

Results

A total of 55 patients with homozygous SCD entered in the study. The mean age was 29.24 ± 9.47 years, and there were 33 (60%) male and 22 (40%) female with the mean BSA of 1.42 ± 0.3 m². The control group had 55 patients with the mean age of 28.82 ± 0.64 years, 34 (62%) male and 21 (38%) female with the BSA of 1.63 ± 0.5 m².

Table 1 shows the clinical and biometric characteristics of patients with SCD compared with the control population. Patients with SCD had a significantly lower BSA of 1.4 ± 0.3 vs. 1.63 ± 0.5, P < 0.05; the serum level of Hb of 8.73 vs. 9.98, P < 0.001; low vitamin D3 level of 21.11 vs. 46.20 ng/mL, P < 0.01; and lower testosterone level of 1.5 ± 0.74 vs. 2.65 ± 1.09 ng/mL. Furthermore, there were high levels of serum ferritin of 317.35 vs. 80.09 ng/L, P < 0.001; high reticulocyte count of 6.11% vs. 2.07%, P < 0.001; high LDH of 485.36 vs. 195.95 U/L, P < 0.001; and high PTH of 68.96 vs. 41.56 pmol/L, P < 0.001.

BMD scan in the SCD group showed normal bone scan in 22 (40%) patients and low BMD detected in 20 (36%) patients with 13 (24%) having very low BMD. The control group showed only two patients with low BMD. The anatomical sites of abnormal bone scan in the SCD group is disclosed in Table 2, with the highest frequency at the lumbar region, n = 18 (55%), followed by distal radius of 10 (30%) and neck of femur of 5 (15%), the lowest at the neck of femur.

Table 3 summarizes disease clinical severity in SCD patients based on frequency of hospitalization and the use of hydroxyurea in relation to BMD status. High clinical severity and abnormal BMD were noted in 16 (29%) patients, whereas 6 (11%) and 11 (20%) patients had moderate and mild clinical severity, respectively.

On the other hand, among patients with normal bone scan, 12 (22%) had high clinical severity, whereas 4 (7%) had moderate severity and 6 (11%) had mild clinical severity with normal BMD, respectively.

Table 4 shows ANOVA analyses comparing the biometric data of three groups of SCD patients based on findings of DXA scan. Comparison was of normal BMD vs. low BMD (osteopenia) and normal BMD vs. very low BMD (osteoporosis).

SCD patients with very low BMD compared with those with normal BMD had significantly higher male ratio, higher LDH level and higher level of PTH, and low BSA with low vitamin D3 and testosterone level. Further, the low BMD group compared with the normal BMD group also showed significant low testosterone level and high LDH enzyme only.

Table 2. Percentage of patients with osteopenia and osteoporosis at different bony sites (n = 33).

| SITE OF ABNORMAL BMD | ABNORMAL BONE MASS DENSITY (BMD) n = 33 |
|----------------------|-----------------------------------------|
| Lumbar (L1–L5)       | 18 (55%)                                |
| Distal radius        | 10 (30%)                                |
| Neck of femur        | 5 (15%)                                 |
In SCD patients with osteoporosis (very low BMD), the mean level of serum vitamin D was lower than 10 ng/mL in 11 (50%) patients, >25 ng/mL in 2 (10%) patients, and >10 and <25 ng/mL in 9 (40%) patients.

Table 5 shows the results of multiple logistic regression analysis for different clinical and biometric variables and predictive risk of abnormal BMD in adult Bahraini SCD patients.

Odds ratio was of significant value for low vitamin D level (<25 ng/mL) of 1.14 (95% CI: 1.042–1.256, P < 0.05), low BSA (<1.2 m²) of 1.1 (CI: 0.85–1.35, P < 0.05), and low testosterone level (<0.9 ng/mL) of 1.2 (CI: 0.91–1.42, P < 0.05). Other variable odds ratio was of no significant predictive value as follow: reticulocyte count 1.19, male sex 1.52, PTH (normal range 18,20–65 pg/mL) 1.02, and LDH (>400 U/L) 0.95.

Discussion
The prevalence of abnormal BMD in adult SCD patients in this study was 33/55 (58%), which is higher than reported by Woods et al, where the percentage of BMD in SCD patients was as low as 32% but lower than the prevalence (72%–79%) found by others. This variation may be because of operational definition of BMD and the ethnic profile of patients included in the study, as patients included in this study were all adults of more than 18 years and were all Bahraini patients, where this disease is endemic.

DXA scan was validated to assess the bone mass density in this study, and it showed the most frequent site of abnormalities at the lumbar spine followed by the radius and the neck of the femur; such finding is in keeping with other studies where the lumbar spine was the most predominant site for the BMD abnormality. Based on the clinical severity of the disease, there were 28 (50%) patients with severe clinical manifestations with more than two hospital admissions per year and with the use of hydroxyurea medication. Those of high clinical severity had the highest rate of abnormal BMD of 29%, which confirms findings reported by Sarrai et al who found that patients using hydroxyurea were more likely to have abnormal BMD.

The serum level of vitamin D3 was significantly low in SCD patients in particular and profoundly lower in those with osteoporosis compared with normal BMD. The above findings was observed by Adam et al where patients were of low height and weight and because of their chronic disease had lack of exercise.

| Table 4. Clinical and biometric data of homozygous SCD patients with low BMD or very low BMD vs. normal BMD (data represented as percentage or mean ± SD). |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age (years)                     | Normal BMD (n = 22) | Low BMD (n = 20) | Very Low BMD (n = 13) | P-value between Normal and Low BMD | P-value between Normal and Very Low BMD |
| Male gender n = 33             | 12 (21%)         | 8 (15%)         | 13 (24%)         | 0.976           | 0.045           |
| Female gender n = 22           | 7 (13%)          | 8 (15%)         | 7 (13%)          | 0.967           | 0.763           |
| BSA (g/dL)                     | 1.43 ± 0.4       | 1.39 ± 0.2      | 1.26 ± 0.2       | 0.732           | 0.05            |
| Hb (g/dL)                      | 8.74 ± 0.75      | 8.74 ± 0.85     | 8.71 ± 0.83      | 0.988           | 0.919           |
| PTH (normal < 65 pg/mL)        | 45.64 ± 3.39     | 48.30 ± 3.36    | 68.62 ± 11.64   | 0.864           | 0.045           |
| Testosterone (ng: 0.2–4.3 ng/ml)| 1.7 ± 0.62       | 1.2 ± 0.72      | 0.792 ± 0.34    | 0.021           | 0.034           |
| VIT D (ng: > 50 ng/mL)         | 24.55 ± 6.72     | 21.30 ± 6.09    | 15.00 ± 4.16    | 0.085           | 0.005           |
| Reticulocyte (%)               | 6.12 ± 0.95      | 6.09 ± 0.99     | 6.17 ± 0.90     | 0.911           | 0.879           |
| Ferritin (ng/L)                | 317.27 ± 97.27   | 321.45 ± 115.71 | 311.15 ± 112.02 | 0.901           | 0.872           |
| LDH (U/L)                      | 347.41 ± 143.36  | 390.80 ± 140.20 | 541.23 ± 133.94 | 0.321           | 0.031           |

Abbreviations: PTH, parathyroid hormone; BMD, bone mineral density; Hb, hemoglobin; LDH, lactate dehydrogenase; BSA, body surface area.
The low level of vitamin D3 in our Bahraini patients with SCD may contribute significantly to the abnormal BMD; such finding was observed by Miller et al.18 Our finding that male gender (60%) was marginally higher than female gender (40%) may be because of the original disease process of homozygous SCD with no female predominance. However, previous studies22–24 of different ethnic origins showed higher female gender.

In this study, the serum level of testosterone was significantly low as follow the low serum level of vitamin D3, both add on an important causative factor of low BMD in patient with SCD compared with the control population.23–25 The concurrent use of opioid derivative in chronic Bahraini SCD patients with high clinical severity of more than two hospital admissions and use of hydroxyurea may be an attributable factor of very low BMD.

The serum level of parathyroid hormone was significantly higher in SCD patients compared to the control group. This finding may be secondary to the very low vitamin D3 status paired with significant low testosterone level.

The raised blood markers of chronic hemolysis, such LDH and reticulocyte in Bahraini SCD patients, indicate high bone marrow turnover and chronic active hemolysis. This is in agreement with Nouraei et al.,26 where the component of direct markers of hemolysis was higher in patients with SCD.

The high serum ferritin level in Bahraini SCD patients compared with the control population is mostly because of repeated blood transfusion; a similar finding was reported in children with SCD.27

This study was conducted in adult Bahraini patients with SCD and no children were enrolled; the increase in patient’s survival and the chronic nature of disease process explain the higher level of serum ferritin.

Limitations of the Study
This study was conducted in adult SCD population but children may have findings that may be important. The small number of the study population makes it difficult to draw concrete conclusion, and a larger number may be necessary for such purpose. The use of T-scores rather than Z-scores in the diagnosis of osteopenia and osteoporosis is another limitation of the study.

Conclusion
The prevalence of abnormal bone mass density (BMD) is high (60%) in Bahraini patients with SCD. There is significant low serum level of vitamin D3 and low testosterone hormone in those with very low body mass density MBD (osteoporosis and T-score <=-2.5). The low serum level of vitamin D3 (<20 ng/mL) and testosterone (<0.9 ng/mL) had risk prediction (odds ratio) of 1.14 and 1.2, respectively, for abnormal BMD in SCD.

Author Contributions
Conceived and designed the experiments: TSG, ABH. Conducted the statistical work: AAJ. Collected data: DSD. Conceived and designed the experiments: TSG, ABH. Conducted the statistical work: AAJ. Collected data: DSD.

Table 5. Multiple regression analysis findings for different predictors of abnormal bone mass density in adult Bahraini patients with homozygous SCD.

| Predictor                  | ODDS RATIO | 95% CI (LOWER-UPPER) | P-VALUE |
|----------------------------|------------|----------------------|---------|
| BSA < 1.2                  | 1.1        | 0.85–1.35            | 0.05    |
| Male gender                | 1.52       | 0.39–5.90            | 0.53    |
| Vitamin D level <20 (ng/ml)| 1.14       | 1.04–1.25            | 0.005   |
| Reticulocyte count (>5%)   | 1.19       | 0.57–2.50            | 0.63    |
| PTH > 65 (pg/ml)           | 1.021      | 0.85–1.27            | 0.87    |
| LDH > 400 (U/L)            | 0.95       | 0.99–1.00            | 0.95    |
| Testosterone < 0.9 ng/ml   | 1.2        | 0.91–1.42            | 0.04    |

Abbreviations: PTH, parathyroid hormone; BMD, bone mass density; LDH, lactate dehydrogenase; BSA, body surface area.

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