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ASSESSMENT OF HEALTH IN PATIENTS WITH SICKLE CELL ANEMIA AND THALASSEMIA: IMPLICATIONS FOR VITAMIN D STATUS AND HEMOLYTIC ANEMIA EFFECTS

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ABSTRACT

Sickle cell anemia and thalassemia are hematological disorders affecting individuals of all ages, potentially impacting bone health, particularly in children. This cross-sectional study assessed bone health in 34 sickle cell patients and 28 patients with hereditary spherocytosis. Various measures including medical history, radiographic imaging, and biochemical analysis were utilized. Results indicated that a significant portion (85%) of participants had insufficient levels of serum 25 OH-vitamin D (<20ng/ml), indicative of inadequate vitamin D supply. Additionally, 31% reported experiencing bone pain. A notable disparity in RANKL/OPG ratios was observed between controls and patients (0.86 ± 0.07 for controls vs. 0.26 ± 0.2 for patients, P = 0.0007). Furthermore, significant differences in osteocalcin levels were noted between healthy controls and patients with osteomalacia. Multiple stepwise regression analysis revealed significant correlations between osteocalcin levels and LDH (partial r2 = 0.29), diagnosis of hemolytic anemia (partial r2 = 0.05), and age (partial r2 = 0.03). Overall, homozygous sickle cell patients exhibited better bone health compared to patients with hereditary spherocytosis. The study underscores the adverse impact of anemia on bone health in children, potentially attributed to osteocalcin deficiency and RANKL/OPG imbalance.

Keywords: Sickle cell anemia, Thalassemia, Bone health, Vitamin D deficiency, Hemolytic anemia.

NTRODUCTION

It results in abnormal hemolysis of erythrocytes when anemia is caused by chronic hemolytic uremic syndrome. A number of factors have been identified as being able to enhance chronic hemolysis [1]. Along with hemoglobinopathy (e.g., sickle cell anemia), enzymopathy refers to anemias resulting from enzyme deficiencies. Autoimmune or microangiopathic diseases can cause intrinsic hemolytic anemia. Sickle cell and thalassemia patients suffer from impaired bone health. The bone mineral density of these patients is abnormal [2–5].

The lack of vitamin D, growth hormones, chronic transfusions, iron toxicity, and diabetes have all been linked to osteoarthritis, as well as other endocrine disorders. It is well known that smoking and inactivity are two of the lifestyle factors that negatively affect bone health. When mice are infected with Plasmodium chabaudi or treated with phenylhydrazine, they suffer from hemolytic anemia that damages their bones [6]. When mice were severely harmed by hemolysis, their bone density decreased and their osteocalcin levels decreased [7].

Recent studies have shown that sickle cell disease patients have higher RANKL/OPG ratios or higher TRAP5b activity. In light of these results, it is possible to conclude that bone remodeling is imbalanced in these individuals. Blood glucose-6-phosphate is deficient in people with hereditary spherocytosis, which enhances the risk of hemolytic anemia. Children with hemolytic anemia were assessed for bone health and bone loss in a cross-sectional study

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MATERIAL AND METHODS

Patients

A total of 45 hemolytic anemia patients (24 women and 21 men) were recruited from the clinic following regular clinic visits. Following regular visits to recruit patients and parents, informed consent was obtained from them. Patients and caregivers signed written consents. Using age-matched control groups and patients with osteoprotegerin levels (OPG), we compared the results. Healthy controls were evaluated as possible endocrine dysfunctional individuals by the endocrine service. Study participants' parents and healthy control group members consented. A total of 17 homozygous sickle cell anemics (HbSS) and 2 sickle hemoglobin C-deficient patients were identified as part of the study, along with six patients with b-thalassemia major and one with b-thalassemia minor, 28 patients with hereditary spherocytosis, two patients with glucose-6-phosphate deficiency, and one with paroxysmal nocturnal hemoglobin-urea patients. In four patients suffering from b-thalassemia major, allogeneic bone marrow transplants were performed. Patient characteristics are summarized in this table.

Tests in laboratories

In addition to lactate dehydrogenase (U/l), bone turnover, pubertal status, and vitamin D metabolism, other biochemical parameters used in the study were potassium and calcium levels. Besides bilirubin, serum alkaline phosphatase and 25-OH vitamin D levels were measured, along with reticulocytes (0/00), 1,25-(OH)2 and 25-OH vitamin D levels (ng/ml). There are several serum parameters in the serum analysis, such as calcium levels (U/l), alkaline phosphatases, BAPs, parathyroid hormone levels (pg/ml), bone mineral density levels, OCs, insulin-like growth factors 1, IGF-1 levels, RANKL levels, OPG levels, and RANKL levels. A spot urine sample along with NTX was examined for deoxypyridinoline (mg/g creatinine) and calcium:creatinine ratio (mg/mg). In this study, SDS values were calculated using Blum and Breier's data regarding serum IGF-1 levels. Serum leptin values were calculated using Mediagnost's (Reutlingen, Germany) software tool for calculating IGF-1 SDS, and BMI and Tanner stage values were used for measuring serum leptin.

Bone densitometry

The BMD was also determined by an anteroposterior view of the lumbar spine in addition to the left femoral neck. According to normative values for the corresponding age group, we calculated Z-scores for lumbar spine measurements. Every patient's BMD was measured by a single investigator without knowledge of their clinical condition.

A Tanner stage assessment was conducted during outpatient clinic visits to determine patients' height, weight, and pubertal status. A digital scale (Seca, Hamburg, Germany) was used to measure the weight to within 0.1 kg. A BMI is calculated by dividing weight (kg) by height (m2). Calculated standard deviation scores were obtained by comparing measurements to a German reference dataset. Tanner stages were used by pediatricians to evaluate pubertal development. A Prader orchidometer was used to measure testicular volume. A standardized questionnaire was also administered, which asked about calcium and vitamin D intake, nutritional supplements, and screen time. Detailed information about the patient's daily diet was collected through patient and parent questionnaires, including milk, sparkling water (what kind), cereal, cheese slices, yogurt, seeds, and fish (what kind). Based on data charts, calculate vitamin D intake. Additionally, patients were asked a series of questions about their back and knee pain, fracture history, and whether or not exercise caused them any discomfort. By analyzing the blood transfusion records of the two years prior to the study visit, the number of transfusions was estimated.

STATISTICS

The Shapiro-Wilk test was used to determine the normality of the data. Alpha 0.1 was used to reject the assumption of a normal distribution. In diagnosis groups (healthy controls, HbSS, and spherocytosis), Kruskal-Wallis tests were used because normal distributions cannot be assumed for most parameters. The Mann-Whitney-U test was used when differences were detected between groups. The Bonferroni-Holm test was used to correct multiple comparisons. To determine LDH levels, stepwise regression analysis was performed after considering age, sex, diagnosis (spherocytosis, HbSS), and LDH levels. A statistical significance level of 0.5000 was achieved for all variables in the model. Relationships between single variables were identified using the Spearman correlation coefficient. Group means and reference cohorts were compared using Mann-Whitney-U tests. It was determined that the data were significant at a significance level of $P<0.05$. The data are described by standard deviation and range. SAS Institute Inc., Carey, NC, USA, performed the analysis using SAS System, version 9.4.

RESULTS

Tables 1 and 2 compare hemolytic anemia in children with and without controls. (Data not shown) There was no significant difference in biochemical or clinical findings between male and female patients. It was determined that 79.6% of patients were in need of vitamin D supplementation (20 ng/ml of serum 25-OH vitamin D), and 43.2% of patients were in need of supplementation at a severe level. A serum vitamin D level of 1 to 30.2 was found in the study. It was determined that the blood level of vitamin D was 49.6619.8 ng/l (range: 18–118). There were 49.1635.9 pg/ml of PTH detected in each blood sample (range: 17.3 to 239.6). Hyperparathyroidism was common among children. The complete list of bone metabolism parameters can be found in Tables 1 and 2. DXA scans

resulted in Z-scores ranging from -20.7461 (22.5 to 0.7, N = 14) for patients who received DXA scans. 14 percent of patients were found to have osteopenia, as indicated by the Z-score of .22. 75-OH vitamin D levels and hemolytic anemia were negatively correlated (r = 0.36, P = 0.03). The osteocalcin level of a healthy adult was 76,6 to 186.1 ng/mL, while the osteocalcin level of an osteoporotic patient was 75651.1 (17.5–247 ng/mL). Osteocalcin levels were below 2.5th per centiles in approximately 16% and 45% of all patients. Most populations had negative IGF-1 SDS values.

NTX levels, DPD levels, leptin levels, and IGF-1 levels are compared to their corresponding norms in terms of SAP values, BAP values, NTX levels, and DPD levels. SAP and BAP levels display a significant correlation (r = 0.94, P 0.0001), as does PTH (r = 0.69, P 0.0001). In both measurement groups and separately, BAP levels were positively correlated with NTX levels (r = 0.90, P = 0.04; n = 5). Patients without hemoglobin storage disease consumed more vitamin D (197 IU/day versus 84 IU/day) than those with HbSS. Between 305 mg and 1984 mg of calcium are consumed by people each day, on average. Approximately three to five hours of active engagement are spent by most participants each day. Furthermore, 12% of patients fractured their bones.

Table 1: An overview of the characteristics and status of bone diseases

All patients (n = 90)		HbSS (n = 34)	Spherocytosis (n = 28)	Healthy controls (n = 28)	P-Value
Female/male	46/44	18/16	14/14	14/16	
Age (years)	9.86±5.5	9.36±4.4	10.45±64	10.3±63.6	0.45
BMI SDS	0.01±2.2	20.11±1.4	0.22±0.76	20.16±1.17	0.32
Pubic hair stage SDS	30.16±0.70	20.28±0.64	0.14±0.42	0.10±1.0	0.38
TV/Breast stage SDS	30.18±0.186	20.20±0.7	0.33±1.0	0.21±61.2	0.43
Height SDS	30.27±0.174	20.17±0.9	0.16±0.6	20.09±2.3	0.39
LDH (U/l)	200±6188.7	569.46±138.4	283.16±42.4	214.56±36.4	,0.0001
Bili (mg/dl)	2.53±61.64	3.08±61.1	3.03±61.9	0.53±60.3	,0.001
Retic ($^{0}/_{00}$)	136.16±107.4	195±693.6	135±6108	NA	*0.08*
25-OH Vit D (ng/ml)	12.6±67.9	9.36±7.4	19.16±5.7	10.86±8.8	0.004
1,25-OH Vit D (pg/ml)	48.4619.8	46.66±13.7	51.5±630.2	NA	*0.73*
SAP (U/l)	216.26±106.7	212.76±84.4	173.2678.8	231.46±57.12	*0.25*
BAP (U/l)	131.46±85.0	124.66±55.1	111.9662.7	NA	*0.002*
PTH (pg/ml)	47.16±34.8	43.76±19.3	37.6615.6	42.266.44	0.45
NTX (nmolBCE/nmol crea)	715.26±648.9	952.56±732 6	117	NA	NA
DPD (mg/g crea)	157.06±77.9	187.26±81.4 12	124.16±70.8	NA	*0.11*
Ca:Crea (mg/mg)	0.07±60.07	0.05±60.04 16	0.08±60.07	NA	0.24
Osteocalcin (ng/ml)	68.56±39.0	45.66±17.6 12	90.06±46.7	115.36±35.2	,0.0001
IGF-1 SDS	20.62±1.2	21.04±1.37	20.36±1.16	1.3±1.3	,0.001
RANKL (pmol/l)	0.87±60.64 17	1.18±60.72 8	0.57±60.47 8	0.29±60.26	0.002
OPG (pmol/l)	3.29±60.55	3.63±60.45	2.93±60.46	3.48±60.64	0.04
DXA (Z-Score)	20.74±1.0	20.6±1.04	20.7	NA	NA

Table 2: Bone-specific alkaline phosphatase, urine deoxypyridinoline, and PTH levels are elevated in patients with bone pain.

	All Patients	HbSS	Spherocytosis
25-OH Vitamin D,20 ng/ml	40.5%	43.7%	31.5%
25-OH Vitamin D,10 ng/ml	30%	40%	0%
BAP/SAP altered	05.6%	13%	0%
PTH elevated	12.5%	11.4%	07.3%

NTX/DPD altered	4.3%	08.7%	0%
Regular back pain	12.4%	20.7%	07.3%
Knee pain with exercise	09.2%	09.2%	3.4.%

There was a significant increase in phosphorus levels among patients suffering from back or knee pain (15,661.6 ng/mL compared to 9,961.6 ng/mL, P0.002). There was a significant difference between those with and without knee pain when it came to serum alkalline phosphatase levels (2798+76.1 U/l, P 0.0016).

A total of 34 individuals were diagnosed with HDSA, while 28 people had spherocytosis. As can be seen in Table 1, there was no difference in age or gender between the two groups. The serum 25-OH vitamin D levels of patients with HbSS were half as high as those with spherocytosis. The level of vitamin D deficiency in HbSS patients is ten ng/ml. The level in patients with spherocytosis is not deficient. Hyperparathyroidism was more common in patients with HbSS, accounting for 22% of cases. Kruskal-Wallis tests showed that sickle cell disease patients had lower osteocalcin levels than healthy controls. A significant difference in RANKL serum levels was observed between sickle cell disease patients and controls (1.1860.72 vs. 0.2960.26 mmol/l, P = 0.002). There were no differences between the two groups in osteoprotegerin (OPG) serum levels. There was a difference of 3,660.4 pmol/l in levels among sickle cell disease patients and spherocytosis patients. A lower level of OPG was observed in patients with spherocytosis (3,560.6 pmol/l, not significantly different) than those with controls. There was a significant difference in the ratio of RANKL to OPG between spherocytosis and sickle cell disease (P = 0.0021). As compared to healthy controls (0.866.07) with RANKL/OPG ratios of 0.3460.24 (P 0.006, Mann-Whitney-U test with Bonferroni-Holm correction), sickle cell anemia patients had the highest ratio (0.3460.24). There was no significant difference between RANKL/OPG ratios between sickle cell disease and spherocytosis patients.

The RANKL/OPG ratios and osteocalcin levels were consistently calculated using age, gender, diagnosis (healthy control, HbSS, or spherocytosis), and LDH in multistep regression models: There was no significant difference in osteocalcin levels between sexes, but there was a significant correlation between osteocalcin levels and LDH, age, and diagnosis within each diagnosis group. There was a significant correlation between dihydrogen phosphate levels, sex, and diagnosis and patient RANKL/OPG levels.

DISCUSSION

It has been found that pediatric hemolytic anemia patients are often vitamin D deficient, based on our tertiary center's experience. 25-OH vitamin D levels were found to be lowest in patients with sickle cell disease. Hemolytic anemia patients with vitamin D deficiency have low calcium stores as well as hyperparathyroidism and a calcium deficiency as a result of vitamin D deficiency. By measuring calcium excretion in urine and 25 OH-Vitamin D levels, looked at the relationship between calcium:creatinine ratios and overall calcium balance in the body. Calcium:creatinine ratios that are low also indicate low 25OH-Vitamin D levels. There was a significantly higher incidence of secondary hyperparathyroidism in patients with severe vitamin D deficiency. Non-treatment of vitamin D deficiency leads to secondary damage to the skeleton in adults and children with sickle cell disease [8-11]. The study [12,13] found that IGF-1 stimulates bone synthesis. In these patients, IGF-1 levels were high. IGF-1 was found to be associated with thalassemia and HbSS in a previous study [14, 15]. IGF-1 levels are low in people with spherocytosis for the first time.

Children often have difficulty assessing their bone health. A bone density measurement (BMD) must be adjusted based on the age and height of the patient. If bone mineral density is below the second standard deviation [16], it is probably an indication of compromised bone health. The BMD assessment, which involves x-rays, was carried out only on children suspected of having bones health impairments in this cohort. Low Z-scores indicate low averages. Despite the fact that reduced BMD is common among sickle cell disease patients and adults, the above-mentioned criteria may contribute to a downward bias in this value.

Injuries to the back and knees can weaken bone structure. Study participants were asked to report self-reported bone pain and biochemical parameters related to bone metabolism in this study. In hemolytic anemia patients with high serum alkaline phosphatase levels, knee pain recurs frequently. There was an association between low vitamin D levels and chronic pain in people with bone pain [17]. Following up on the previous study, the same group found that patients with sickle cell diseases who take vitamin D supplements experience less chronic pain.

A comparison of sporocytotic homozygous sickle cell disease and sickle cell leukemia

The vitamin D3 intake in sickle cell anemia patients is slightly higher than in those with spherocytosis, but this is significantly different. Because primary care physicians provide different nutritional recommendations, vitamin D3 intake levels may vary. In Germany, the daily recommended amount of vitamin D3 is only 1 IU per day, which is less than the amount even sickle cell disease patients consumed.

Bone formation and resorption are required for physiological modeling and remodeling.

Hemolytic anemia patients had significantly lower osteocalcin levels than healthy controls. Previously, hemolytic anemia in conjunction with b-thalassemia and sickle cell disease has been linked to a deficit in bone formation. A significant difference was found between hemolytic anemia patients and controls in their RANKL/OPG ratios. By binding to RANKL's receptor, OPG stimulates osteoclasts and inactivates RANKL.

In sickle cell and spherocytosis patients, osteocalcin and RANK/OPG levels were significantly different after one-way ANOVA was applied. The osteocalcin levels of sickle cell patients were significantly higher than those of other groups. In comparison with healthy individuals, sickle cell patients had a significant difference in their RANK/OPG ratios. RANK pathway activation can result in osteoclast activation in sickle cell disease due to chronic inflammation. As a result of sickle cell disease, the osteoclast marker TRAP5b is elevated in adults. When patients with sickle cell disease suffer a severe hemolysis, they may also lose more bone. The results of multistep regression indicate a link between RANK/OPG levels and serum LDH levels in osteocalcin-deficient patients.

CONCLUSIONS

Several similarities exist between hemolytic anemia and impaired bone health, the findings of comparable studies have improved our understanding of sickle cell disease and thalassemia patients as a whole. It is more common for sickle cell disease to affect the bone health than spherocytosis. A person's phenotype of bone pathology can be affected by the pathogenesis and severity of hemolysis. Further research on bone health in hemolytic disease patients is recommended in this report. An evaluation of bone health is recommended for patients with these conditions.

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