The effects of fetal hemoglobin levels on sickle cell anemia patients bone biochemistry

Fetal hemoglobin seviyesinin orak hücre anemisi hastalarının kemik biyokimyası üzerine etkisi

Abstract: Objective: The aim of this study was to evaluate the possible roles of fetal hemoglobin levels on bone parameters in sickle cell anemia (SCA) patients.

Methods: Blood samples taken from 56 SS and 47 control totally 103 subjects were included in this research work according to their fetal hemoglobin levels. Fetal hemoglobin, bone mineral density, bone specific alkaline phosphatase, calcium, osteocalcin, 25-OH vitamin D and hematological parameters were measured and analyzed in the study.

Results: Statistical analysis showed that, lower bone mineral density and biochemical bone parameters significantly correlated with low fetal hemoglobin levels at SCA patients.

Conclusion: It was concluded that fetal hemoglobin level is a good index for bone status in sickle cell anemia patients.

Keywords: Bone, Fetal hemoglobin, Sickle cell, Anemia

1 Introduction

Sickle cell anemia (SCA) is one of the well known autosomal recessive hereditary disease in the world which is characterized by a chronic hemolytic anemia due to the shortened lifespan of the sickle-shaped red blood cells [1,2]. It is known that sickle-shaped red blood cells cause vascular occlusion, leading to tissue ischemia and infarction, known as vascular occlusive crisis [3–5].

Fetal hemoglobin (HbF) is the major hemoglobin present during gestation. It constitutes approximately 60 to 80 percent of total hemoglobin in the newborn [1,6]. It is almost completely replaced by adult hemoglobin during 6 to 12 months of age; and its amounts decreases to less than 2% of total hemoglobin in the adult [7].

HbF inhibits deoxygenation-induced polymerization luğu, kemik spesifik alkalen fosfataz, kalsiyum, osteokal- sin, 25-OH vitamin D ve hematolojik parametreler analiz edildi.

Bulgular: İstatistiksel analizler, düşük kemik mineral yoğunluğu ve biyokimyasal kemik parametrelerinin orak hücre anemisi hastalarında fetal hemoglobin seviyeleri ile yakından ilişkili olduğunu göstermiştir.

Sonuç: Orak hücre anemisi hastalarının kemik durumunu için fetal hemoglobin seviyesinin iyi bir parametre olduğu belirlenmiştir.

Anahtar Kelimeler: Kemik, Fetal hemoglobin, Orak hücre, Anemi
of HbS that drives the pathophysiology of SCA [1,7]. The distribution of HbF concentrations per F cell (HbF containing cell) is likely to be a critical determinant of the protective effect of HbF in SCA [8,9].

SCA patients show a variety of bone disorders including bone pain, bone deformity, bone age delay, growth failure, rickets, scoliosis, spinal deformities, nerve compression, pathologic fracture and osteopathy [10]. Osteopathy is characterized by low bone mass and disruption of bone architecture, resulting in reduced bone strength and increased risk of fractures [4,11].

There is limited information in the literature about the relationship between bone mineral density (BMD) and growth, and other clinical and laboratory findings in SCA patients [12,13]. Gaining more insights into the processes and influencing factors in bone formation and resorption in patients with SCA in different stages of life may help in finding interventions for preserving skeletal health [14–17].

In this study, we investigated the effect of hemoglobin F levels on bone status in SCA patients.

2 Materials and Methods

2.1 Subjects

In this study 56 SCA patients (SS), who attended to the Medical Biochemistry Department at Cukurova University Hospital and determined as SCA by molecular analysis methods [18], was choosen as a patients group (Group I) and 47 healthy persons as a control (Control Group). These patients of homozygosity of sickle cell anemia do not have transfusion and HbF inducing agent therapy history. Group I was divided into two subgroups and classified according to the HbF levels observed in this study (Group II [HbF ≤2%]) and the (Group III [HbF ≥2%]). An informed consent was obtained from patients and it was observed that none of these patients had classical risk factors for osteopathies (no family history of bone fracture, no alcohol abuse, no corticosteroid treatment).

2.2 Bone mineral density

Subjects were scanned for BMD and bone mineral content (BMC) at anteroposterior lumbar spine (L1-L4) and femoral neck, by using dual energy X-ray absorptiometry (LUNAR DPXMD#7164), which was daily calibrated according to manufacturer’s instructions. The BMD and BMC results were respectively expressed as mean values (g/cm²)±SD and (g)±SD, and as a T-score (difference in SD from healthy age matched subjects) [10].

2.3 Biochemical parameters

Venous blood samples (6 mL) were taken from patients and controls into the tubes with EDTA for HbF and hematological analysis and gel vacuum tube was used for alkaline phosphatase, calcium, osteocalcin and 1-25-OH vitamin D analysis.

Fetal hemoglobin and 25-(OH) vitamin D 25-(OH)D levels were measured by using high performance liquid chromatography (HPLC) [4,14]. Bone specific alkaline phosphatase concentration was performed by using Bessey-Lowry-Brock’s technique. Measurement of calcium was performed by using indirect potansiometry technique and osteocalcin was performed by using electrochemiluminescence immunoassay technique [19].

2.4 Statistical analysis

All descriptives were given in mean-standard deviation. Analysis of differences in parameters were made two phases: I) Controls and patients subgroups were analyzed using analysis of variance and post-hoc evaluations were made by the use of Dunnet test. II) Control and “all patients” were analyzed using the student – t test. For all statistical evaluation critical significance level was set at α=0.05.

3 Results

The clinical features, laboratory and Bone Mineral Density (BMD) datas of the groups were summarized at Table 1. According to the Table 1, all groups did not differ from each other in terms of age, sex, height and Body Mass Index (BMI).

All of the biochemical bone markers were found to be significantly different between controls and patient groups.

Osteocalcin values were highest controls, and lowest patients with low HbF (F=52.516, p<0.001)

These values were also found to differ significantly between controls and patients in total (t=6.621, p<0.001)

Vitamin D values were highest controls, and lowest patients with low HbF (F=33.94, p<0.001)

These values were also found to differ significantly
between controls and patients in total (t=7.141, p<0.001)

BSAP values were lowest controls, and highest patients with low HbF (F=125.168, p<0.001)

These values were also found to differ significantly between controls and patients in total (t=8.831, p<0.001)

Calcium values were highest controls, and lowest patients with low HbF (F=6.587, p=0.002)

These values were not found to differ significantly between controls and patients in total (t=3.554, p=0.571)

Serum osteocalcin and vitamin D levels in all patients groups (Group I-III) were found lower than the Control Group (Table 1). These differences between Control Group and Groups I and II and the differences between Group II and Group III were observed significant while the differences between the Control Group and Group III and the differences between group I and II were found nonsignificant.

In addition to osteocalcin, the other bone status marker serum Ca level was found significantly lower for all patients group according to the Control Group (Table 1).

In contrast to these markers, Bone Specific Alkaline Phosphatase (BALP) levels for all patients groups were found higher than the Control Group and the significance were observed for Group I and II according to the Control Group (Table 1).

The BMD of the examined subjects was expressed as the Z-score according to the WHO (World Health Organization) classification [20]. A large majority of the patients showed bone mass below one standard deviation from the mean value of the healthy subjects. Together, 29 out of 56 subjects with SCA major showed osteopathy of the spine and 27 out of 56 had low bone mass at the femoral neck. Bone mass was lower in Group 1 than in Group 2 with a more prevalent osteopathy of the spine in patients. Group 1 had a lower bone mass than Group 2 (p<0.05).

4 Discussion

The sickle cell disease (SCD) is a genetic disorder causing anemia and acute and chronic tissue damage in multiple organs [14]. The bone is one of the main targets of SCD [12,21]. The bone in SCD is affected by osteopenia, osteoporosis, osteomyelitis and osteonecrosis. In particular osteopathies like spontaneous bone fractures seems to be present in a variable percentage of the SCA patients [4].

The studies have showed that same factors such as the level of HbF modulated the severity of the symptoms of the SCD [7]. It was observed that the high level of HbF (above 2%) in SCD patients caused the risk reduction [12].

In this study we compared bone turnover markers such as osteocalcin, vitamin D, calcium and bone specific alkaline phosphatase levels for osteopathy and BMD between the SCD patients and controls.

We found that except BSAP levels, the other bone parameter levels was found lower than the control levels for SCD which were in agree with the other studies [4,14,22].
It is known that vitamin D is a major modulator of calcium homeostasis [4,23]. The deficiency of vitamin D reflects the low calcium levels. Also many studies were described that calcium levels in SCA markedly decreased [4,10,14,24]. In our study we also found a significantly low level of vitamin D and calcium for SCA patients according to the healthy control (Table 1, Table 2, Figure 1) and the lowest level was found for group II (HbF ≤2%). For group III (HbF ≥2%) the level of calcium and vitamin D still lower than control but higher than Group I and II.

It was obvious both vitamin D and calcium are required for optimal bone health. The absorption of sunlight is necessary for the synthesis of vitamin D [14].

With some reasons such as impaired exposure to sunlight, pigmentation like in individuals of Africa, in pale skinned people, inadequate intake, and especially hemolysis, inflammation, the role of the synthesis of vitamin D deficiency caused low calcium levels [4].

Adewoye et all. Have shown that treatment of adult SCD with vitamin D and calcium can restore 25-(OH)D levels to normal and improved BMD [14].

Low BMD is related in part to increased bone resorption [13].

In analogy with thalassemia, bone loss in SCD has been ascribed in part to bone marrow hyperplasia secondary to chronic anemia and SCD individuals delays in skeletal maturation which may affect bone density the low level of osteocalcin has been suggesting a role for decrease bone formation in the BMD deficiency [11,22,25].

In this study SCA patients displayed significantly lower levels of osteocalcin (a marker of bone formation) than the healthy control group. Again the higher values of osteocalcin in group II according to the group II means that some restoration about the vitamin D and calcium levels secondarily affect the osteocalcin levels.

It was found the recurring knee pain was associated with higher serum bone specific alkaline phosphatase levels in patients with hemolytic anemia.

In our study the BSAP levels for all groups were found higher than control group. We know that BSAP is bone formation markers and low bone formation rather than high bone resorption may explain the bone mineralization [17,19].

Increased bone catabolism and diminished bone mineralization occur as a result of various genetic and acquired factors in SCA patients. DXA is considered a gold standard for BMD assessment. In the present study, lumbar and femoral BMD values of the patients with group I were found significantly lower. It has been reported that SCA have low Z-score value at lumbar spine compared to femur. This may be explained in terms of the nature of SCA where trabecular bones such as lumbar vertebrae are mainly affected by bone marrow expansion because of ineffective hematopoiesis. In the light of recent findings, it was thought that the significant decrease in the BMD values of lumber vertebrae in our patients was secondary to the disease. In the light of our results, it can be concluded that decreased BMD is a risk factor in osteopathies.

As a result in this study it was observed that all bone markers value is better for HbF ≥2% cases than HbF ≤2% cases. This risk reduction may depends on the inhibition of deoxygenation-induced polymerization by high degree of HbF levels which is indirectly decreasing the hemolysis of the red blood cell.

|                | Control groups | All patients | Patients with low HbF | Patients with high HbF |
|----------------|----------------|-------------|-----------------------|-----------------------|
| Osteocalcin    | 16.3±**        | 13.2±3.4*   | 10.9±2.3*             | 15.8±1.9*             |
| 25-(OH)D       | 14.8±1.9*      | 12.3±1.8*   | 11.2±1.4*             | 13.3±1.1*             |
| BALP           | 13.3±1.1*      | 23.7±5.9*   | 27.3±2.6*             | 18.2±4.9*             |
| Calcium        | 9.21±1.4*      | 8.3±1.5     | 8.2±1.3*              | 8.4±1.7*              |

*Analysis of variance shows significant difference (P<0.002). +Student t test shows significant difference (P<0.001).
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5 References

[1] Colella MP, Traina F. Fetal hemoglobin and hemolysis markers in sickle cell anemia. Rev Bras Hematol Hemoter 2015; 37(3):148–9.
[2] Adewoyin AS. Management of sickle cell disease: a review for physician education in Nigeria (sub-saharan Africa). Anemia 2015; 2015(791498).
[3] Alvarez C, Silva de Roldan FN, Negron JC. Bone scan in sickle cell disease. Bol Asoc Med P R 1984; 76(11):477–8.
[4] Arlet JB, Courbebaisse M, Chatellier G, Eladari D, Souberbielle J C, et al. Relationship between vitamin D deficiency and bone fragility in sickle cell disease: a cohort study of 56 adults. Bone 2013; 52(1):206–11.
[5] Darbari DS, Ballas SK, Clauw DJ. Thinking beyond sickling to better understand pain in sickle cell disease. Eur J Haematol 2014; 93(2):89–95.
[6] Ahmadvand M, Noruzinia M, Fard AD, Zohour MM, Tabataibafar MA, et al. The role of epigenetics in the induction of fetal hemoglobin: a combination therapy approach. Int J Hematol Oncol Stem Cell Res 2014; 8(1):9–14.
[7] Moreira JA, Laurentino MR, Machado RP, Barbosa MC, Goncalves RP, et al. Pattern of hemolysis parameters and association with fetal hemoglobin in sickle cell anemia patients in steady state. Rev Bras Hematol Hemoter 2015; 37(3):167–71.
[8] Paciaroni K, Gallucci C, de Angelis GC, Alfieri C, Roveda A, et al. Sustained and full fetal hemoglobin production after failure of bone marrow transplant in a patient homozygous for beta 0-thalassemia: a clinical remission despite genetic disease and transplant rejection. Am J Hematol 2009; 84(6):372–3.
[9] Papayannopoulou TH, Brice MS. Stimulation of fetal hemoglobin synthesis in bone marrow cultures from adult individuals. Proc Natl Acad Sci U S A 1976; 73(6):2033–7.
[10] Garrido Colino C, Belendez Bieler C, Perez Diaz MC,ela de Julian E. Evaluation of bone mineral density in children with sickle cell disease. An Pediatr (Barc) 2015; 82(4):213–21.
[11] Alavi A, Heyman SK, Kim HC. Scintigraphic examination of bone and marrow infarcts in sickle cell disorders. Semin Roentgenol 1987; 22(3):213–24.
[12] Garadah T S, Hassan AB, Jaradat AA, Diab DE, Kalafalla HO, et al. Predictors of abnormal bone mass density in adult patients with homozygous sickle-cell disease. Clin Med Insights Endocrinol Diabetes 2015; 8(35–40).
[13] Meeuwes M, Souza de Carvalho TF, Cipolotti R, Gurgel RQ, Ferrao TO, et al. Bone mineral density, growth, pubertal development and other parameters in Brazilian children and young adults with sickle cell anemia. Trop Med Int Health 2013; 18(12):1539–46.
[14] Adewoye AH, Chen TC, Ma Q, McMahon L, Mathieu J, et al. Sickle cell bone disease: response to vitamin D and calcium. Am J Hematol 2008; 83(4):271–4.
[15] Omojola MF, Annobil S, Adzaku F, Addae SK, Mohammed S. Bone changes in sickle cell anemia. East Afr Med J 1993; 70(3):154–8.
[16] Spina-Castro AM, Siaviero-Miacohon AA. Low bone mineral density in patients with Sickle Cell Anemia (SCA) and short stature should be interpreted with caution. Trop Med Int Health 2014; 19(3):364–5.
[17] Voskaridou E, Kyrtsos M, Terpos E, Skordili M, Theodoropoulos I, et al. Bone resorption is increased in young adults with thalassemia major. Br J Haematol 2001; 112(1):36–41.
[18] Attila G, Yalin S, Tuli A, Yalin E. Prenatal diagnosis of sickle cell anemia in twin pregnancies and identification by VNTRs. Clinica Chimica Acta 2004; 350(1–2):137–142.
[19] Azinge EC, Bolarin DM. Osteocalcin and bone-specific alkaline phosphatase in sickle cell haemoglobinopathies. Niger J Physiol Sci 2006; 21(1–2):21–5.
[20] Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. Osteoporos Int 1994; 4(6):368–81.
[21] Osunkwo I. An update on the recent literature on sickle cell bone disease. Curr Opin Endocrinol Diabetes Obes 2013; 20(6):539–46.
[22] Aguilar C, Vichinsky E. Bone and joint disease in sickle cell disease. Hematol Oncol Clin North Am 2005; 19(5):929–41, viii.
[23] Weiss E, Rees DG. Gibson JS. Role of calcium in phosphatidyserine externalisation in red blood cells from sickle cell patients. Anemia 2011; 2011(379894).
[24] Oliveira JF, Vicente NG, Santos JP, Weffort VR. Vitamin D in children and adolescents with sickle cell disease: an integrative review. Rev Paul Pediatr 2015.
[25] Koguma M, Matsuda K, Okuyama R, Yanai NO, Mina M. Selective proliferation of lymphoid cells from lineage-c-Kit+ Sca-1+ cells by a clonal bone marrow stromal cell line. Exp Hematol 1998; 26(4):280–7.