Influence of androgens on bone mass in young women with sickle cell anemia

Abdulmohsen H. Al-Elq, MBBS, ABIM, *Haifa A. Al-Turki,* SSC , ABOG, *Osama A. Sultan,* MBBS, KFUF, *Mir Sadat-Ali,* MS, FRCS.

ABSTRACT

الأهداف: لتقييم العلاقة بين مستوى الهرمونات الجنسية وكثافة العظام لدى النساء في سن ما قبل اليأس، والمصابات بالأنيميا المنجلية.

الطريقة: شملت هذه الدراسة المقطعية النساء البالغات والمصابات بالأنيميا المنجلية واللاتي حضرن إلى عيادة أمراض الدم أو عيادة العظام في مستشفى الملك فهد الجامعي – الخبر – المملكة العربية السعودية، في الفترة مابين أغسطس 2006م وحتى يونيو 2007م. بعد أخذ الموافقة للمشاركة في هذه الدراسة، أجري فحص إكلينيكي للمريضات، وقمنا بتدوين العمر وحساب مؤشر كثافة الجسم (BMI). تم سحب عينات دم مختلفة بما فيها عينة لقياس هرمونات التأنيث (E2) وهرمون التذكير (Te). وتم قياس كثافة العظام (BMD) لجميع المريضات في منطقة العامود الفقري وعظمة الفخذ بواسطة جهاز (DEXA).

النتائج: حُللت بيانات 51 مريضة بمتوسط عمري 26±3.1 عام، وتم تقسيم المرضى إلى مجموعتين (مجموعة أ ومجموعة ب). مجموعة (أ) لديها كثافة عظام (BMD) طبيعية وتحوي 31 مريضة (60.8%)، ومجموعة (ب) لديها كثافة عظام (BMD) منخفضة وتحوي 20 مريضة (39.2%). كانت نتيجة مستوى هرمون التأنيث متساوية لدى المجموعتين، أما مستوى هرمون التذكير فكان أقل لدى المريضات اللاتي لديهن كثافة عظام منخفضة (38±11.8 versus 22.3±11.7 ng/dl, $p<0.001$).

خاتمة: دلت هذه الدراسة على أن هرمون التذكير قد يلعب دوراً هاماً للمحافظة على كثافة العظام (BMD) لدى النساء المصابات بمرض الأنيميا المنجلية في عمر ما قبل سن اليأس.

Objective: To evaluate the relationship between the gender hormonal levels and bone mineral density in premenopausal women suffering with sickle cell disease.

Methods: A cross-sectional study including consecutive female adult patients with sickle cell anemia attending the outpatient hematology/orthopaedic clinics, or admitted to King Fahd University Hospital, Al-Khobar, Saudi Arabia, between August 2006 and June 2007. Patient's age was documented, and body mass index was calculated. Blood was drawn for complete blood picture, biochemistry, and hormonal profile including total estradiol (E2) and total testosterone (Te). Bone mineral density (BMD) was measured for all patients using dual energy x-ray absorptiometry scan at the hip and lumbar spine.

Results: We analyzed the data of 51 patients with an average age of 26±3.1 years. Patients were divided into 2 groups (group A and group B). Group A had normal BMD and group B with low BMD. Thirty-one (60.8%) were in group A and 20 (39.2%) were in group B. The E2 level was not statistically different between the 2 groups, while Te level was significantly lower in women with low BMD (38±11.8 versus 22.3±11.7 ng/dl, $p<0.001$).

Conclusions: Our study indicates that in premenopausal female patients with sickle cell anemia, testosterone may play a role in the preservation of bone mass.

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From the Departments of Internal Medicine (Al-Elq, Sultan), Obstetrics & Gynecology (Al-Turki), and Orthopedic Surgery (Sadat-Ali), College of Medicine, King Faisal University, Dammam, & King Fahd University Hospital, Al-Khobar, Kingdom of Saudi Arabia.

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Address correspondence and reprint request to: Prof. Mir Sadat-Ali, PO Box 40071, King Fahd University Hospital, Al-Khobar 31952, Kingdom of Saudi Arabia. Tel. +966 505848281. Fax. + 966 (3) 8971013. E-mail: drsadat@hotmail.com

Androgens, particularly testosterone, are the main determinant of male skeletal health.[1] An age related decline of testosterone levels and the risk of osteoporosis and osteoporotic fractures in men has been clearly identified.[2,3] Recently, estrogen was found to play an important role in the development of male osteoporosis, and in some reports it was considered to be more important than the androgens.[4,5] However, loss of estrogen at menopause causes osteoporosis in many

women.[6] As in men, women experience a decrease in the androgen levels with time. A consensus statement in 2002, described a state of "androgen insufficiency syndrome," in which low levels of androgen can adversely affect women's health including the bone.[7] Slemenda et al[8] studied 231 women between the ages of 32-77 years with multiple measurements of gender steroids over 2-8 years, and found the bone loss to be significantly associated with lower androgen concentration in premenopausal women, and with lower estrogens and androgens in peri-and postmenopausal women. Also, studies have demonstrated that the combination of androgens and estrogen replacement therapy in postmenopausal women increased bone mass to a greater extent than estrogen alone.[9,10] From the above, it appears that both estrogen and androgen hormones are important for the reservation of bone mass in men and women. Males and females with sickle cell anemia (SCA) are known to suffer from secondary osteoporosis,[11-13] and still there is no consensus for a cost-effective laboratory test. Several mechanisms were postulated for the development of osteoporosis in these patients. As the risk of development of hypogonadism in patients with SCA is small, as compared to patients with other hemoglobinopathies such as thalassemia,[14] we hypothesize that low androgen levels can be one of the causes of low bone mass in such patients. This study was carried out with the aim to find the relationship of testosterone levels in the development of low bone mass in patients with SCA at the age of peak bone mass.

Methods. This is a cross-sectional study including consecutive adult female patients with SCA aged 25-35 years (age of peak bone mass) attending the outpatient hematology clinic, or admitted to King Fahd University Hospital, Al-Khobar, Saudi Arabia, between August 2006 and June 2007. The study was approved by the Research Committee of the College of Medicine, King Faisal University, Dammam, Saudi Arabia. Patients with hemoglobinopathies other than homozygous disease were excluded. Other exclusion criteria were clinical or laboratory evidence of secondary causes of osteoporosis apart from the original disease, pregnant and lactating women, patients with bilateral femoral head replacement, prolonged immobilization, significant organ dysfunction, and patients who were already diagnosed or treated for osteomalacia and low bone mass. After informed consent to participate, clinical evaluation was carried out by one of the authors. Every effort was made to rule out other causes of secondary osteoporosis and none of our patients had oophorectomy, chemotherapy, or radiotherapy. Also, none of them were on anti androgen therapy or corticosteroids. Patient's age was documented, and body mass index (BMI) was calculated. Blood was drawn in the morning for complete blood picture, and hemoglobin electrophoresis. Other tests collected were renal function test, liver function test, calcium, phosphorus, alkaline phosphatase, leuteinizing hormone (LH), follicle stimulating hormone, (FSH) total estradiol (E2), and total testosterone (Te). The Te and E2 were analyzed using Architeci 2000 machine using the chemiluminescent microparticle immuno-assay. Bone mineral density (BMD) was measured at 2 sites, at the lumbar spine (L1-L4) and at the proximal femur using dual energy x-ray absorptiometry (DEXA) scan, (Hologic Inc. Walthan, MA, USA). A standard position was used for anterior-posterior scan of the lumbar and the proximal femur. Standard deviation (SD) from the young adult normal mean (T-score), and from the aged matched mean adjusted for body weight (Z-score) were documented. Bone mineral density results were expressed as gm/cm^2. Patients with T-score of less than 2.5 SD below the norm were labeled to be osteoporotic and those having a T-score between 1-2.5 SD below the norm were labeled to be osteopenic as per the WHO criteria. Since our patients are at the premenopausal age group, and due to the sample size, those with osteoporosis and osteopenia were grouped together and labeled as having low BMD during the analysis.

The data were entered in the database and analyzed using SPSS (Statistical Package for the Social Sciences, Chicago, Illinois). Means were compared using students "t" test and Chi-square as needed. Statistical significance of $p<0.05$ with confidence interval of 95% was used during the analysis.

Results. We analyzed the data of 51 patients with an average age of 26±3.1 years. Patients were divided into 2 groups (group A and group B). Group A has normal BMD and group B with low BMD. Thirty-one (60.8%) were in group A and 20 (39.2%) were in group B. At the hip region the BMD was 0.964 ± 0.13 gm/cm^2 in group A, versus 0.640 ± 0.20 gm/cm^2 (?not the same as **table 2**) in group B (p=0.01), while the mean T score was 0.1 ± 0.7 versus -3.1 ± 0.48 in group B (p=0.01). The results of BMD and T score of lumbar spine in the women of group A were significantly higher when compared to women of group B (p=0.01). The average age was similar in the 2 groups with a non-significant p-value (p=0.3). The difference in the BMI was also not significant between the 2 groups (p=0.1). Hematological and biochemical parameters were within the normal range and there was no statistically significant difference between both groups (**Table 1**). Regarding the hormonal profile (**Table 2**), LH and FSH were similar in the 2

Table 1 - Demographic data, hematological and biochemical parameters of both group.

Parameter	Group A	Group B	*P*-value
Number of Patients	**31**	**20**	
Age (years)	26 ± 3.7 (25-35)	27 ± 1.50 (25-34)	0.1
BMI kg/m^2	17.82 ± 3.38	17.4 ± 4.9	0.1
% Hemoglobin "S"	85.2 ± 4.56	87.1 ± 3.9	0.1
Hemoglobin concentration (g/dl)	9.51 ± 1.01	9.52 ± 1.02	0.1
Blood urea nitrogen 7-22 mg/dl)	8.54 ± 3.5	7.81 ± 2.19	0.3
Creatinine (0.5-1.0 mg/dl)	0.52 ± 0.11	0.48 ± 0.13	0.8
Serum calcium (8.5-11.5 mg/dl)	8.8 ± 0.51	8.7 ± 0.58	0.8
Phosphorus (2.5-4.9 mg/dl)	3.65 ± 0.58	3.44 ± 0.62	0.2
Alkaline phosphatase <270 u/l)	102.8 ± 44.3 (70-179)	107.3 ± 42.6 (40-496)	0.7

Values are expressed as means ± SD, BMI - body mass index

Table 2 - Hormonal levels and BMD of hip and spine of group A and B.

Parameter	Group A	Group B	*P*-value
Hip BMD gm/cm^2	0.964 ± 0.13 (0.844 - 0.21)	0.640 ± 0.20 (0.469 - 0.97)	0.01
T Score	0.1 ± 0.7 (-0.1 - 1.3)	-3.1 ± 0.48 (-2.6 - -4.2)	0.01
Z Score	-0.1 ± 0.8 (-0.4 - 1.6)	-2.0 ± 1.39 (-1.2 - -3.8)	0.01
Spine BMD gm/cm^2	0.950 ± 0.15 (0.780 - 1.27)	0.739 ± 0.23 (0.560 - 1.11)	0.01
T Score	-0.5 ± 1.4 (-0.6 - -1.2)	-3.5 ± 1.32 (-1.6 - -5.4)	0.01
Z Score	-0.97 ± 1.4 (1 - -1.7)	-3.0 ± 1.35 (-1.1 - -5.2)	0.01
LH (1.26-10 MIU/ml)	10.6 ± 22.9 (1.53 - 72.6)	4 ± 2.81 (0.52 - 9.01)	0.01
FSH (1.37-13.58, MIU/ml)	4.46 ± 2.1 (0.72 - 8.04)	4.01 ± 2.23 (1.53 - 8.78)	0.4
Total estradiol (11-44 pg/dl)	52 ± 22.6 (22.8 - 82.7)	47.5 ± 19.1 (10.4 - 75.1)	0.1
Total testosterone (9-109 ng/dl)	38 ± 11.80 (10.6 - 67.2)	22.3 ± 11.7 (8 - 44)	0.001

Values are expressed as means± SD, BMD - bone mineral density, LH - leutinizing hormone, FSH - follicle stimulating hormone

groups, while LH was significantly higher in patients with normal BMD. There was no statistically difference between the 2 groups in terms of E2 level (p=0.1). The Te level was significantly lower in women with low BMD (p<0.001).

Discussion. Adult patients with SCA were found recently to have a high incidence of osteopenia and osteoporosis. The reported incidence of low bone mass is up to 79.6%.[11] The low incidence found in this study (39.2%) is most likely related to the age group we studied (25-35 years), which is an age of peak bone mass. In a previous study, we found an incidence of low bone mass in 79% among patients aged 26-47 years.[12] Several mechanisms were postulated to be the cause of the osteopenia and osteoporosis in patients with SCA including erythroid hyperplasia, altered secretion of growth factors, and vitamin D deficiency.[15-17] Hypogonadism should be considered as one of the causes of low bone mass in these patients related to iron overload or ischemia,[18] however, the recently reported incidence of hypogonadism in patients with SCA is low,[14] and in fact none of our patients were documented to have hypogonadism. Since the E2 level is normal in the group of low bone mass, it is unlikely that the non-significant difference in the estradiol level between the 2 groups contributed to the difference in the BMD. Interestingly, we found a highly significant difference in the Te level between the 2 groups (p<0.001). Androgens were found recently to be important in the reservation of bone mass in females,[19,20] including the premenopausal age group.[8,21,22] In women, both adrenals and ovaries play a major role in the biosynthesis of androgens. Of all androgens produced by the female, approximately 50% come from the adrenal source, 20-25% come from the ovaries, and 25-30% by peripheral conversion in the adipose tissue. Serum androgen levels in females decline steeply with age, and this decline starts in the early reproductive age leading to a state of androgen insufficiency. The diagnosis of female androgen insufficiency is difficult to objectively quantify due to lack of a sensitive assay that detects low levels of androgens.[7,23,24] Reviewing the English literature, we could not find any study that evaluated the androgen level in female patients with SCA. We have no specific explanation for the difference in the testosterone level between the 2 groups, however, the finding of this study highlights the possibility of a role for the androgens in the preservation of bone mass in patients with SCA. Low androgen levels can be one of the mechanisms for the high incidence of low bone mass in such patients. Although there was no significant difference in the BMI between the 2 groups, patients in both groups have low BMI.

Our study draws attention to an important observation, however, it has some limitations including the fact that we measured the Te and not the free or the "bioavilable" testosterone. Also, we did not evaluate the other androgens, such as dehydroepiandrosterone sulfate and androstenedione. In addition, although all the blood samples were withdrawn in the morning, no

consideration was taken to the phase of the menstrual cycle, keeping in mind the fact that the androgens are highest at the middle third of the cycle. Further and larger studies with avoidance of the above limitations are needed. Also it will be important in future studies to have a control group to prove that this phenomenon is related to SCA. Confirmation of this finding will help in the management of low bone mass in these patients. Testosterone was found to enhance the effect of estradiol on bone density.[9]

In conclusion, our study showed that total serum testosterone level is lower in young female patients with SCA having low BMD compared to patients with normal BMD. This may indicate a possible role for the androgens in the preservation of bone mass in such patients. Further studies are needed for the confirmation of this finding.

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