Bone Structure-Related Biomarkers in Hemophilic Patients, Compared to Healthy Condition

Alireza Abdollahi1,2, Gholamreza Toogebr2, Sahba Gharechahi1, Katayoun Ziar3

1 Department of Pathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2 Thrombosis Hemostasis Research Center, Tehran University of Medical Sciences, Tehran, Iran
3 Department of Pathology, AJA University of Medical Sciences, Tehran, Iran

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Abstract- The increased risk for developing loss of bone density in patients with hemophilia has been recently regarded. The present study was conducted to compare the levels of vitamin D and other biochemical factors affecting bone turnover in patients with hemophilia and those without this problem. The study participants were stratified into the following subgroups 1) the hemophilic patients without evidence of viral infections, 2) those with the healthy condition without evidence of infection by the viral infections. All subjects were asked to take venous blood sample to assess the levels of serum biomarkers related to bone metabolism and turnover. Comparison of different biochemical markers related to bone metabolism and turnover showed significantly lower free testosterone, total testosterone, thyroid stimulating hormone (TSH), vitamin D, calcium, osteocalcin, calcitonin, and parathormone levels as well as higher serum alkaline phosphatase, Serum C-telopeptide (CTX), and N-terminal telopeptide (NTx) levels in those hemophilic patients as compared to control group. Hemophilia can adversely affect bone structure leading to bone loss in hemophilic patients. An appropriate screening protocol pertaining to osteoporosis must be implemented in the facilities for hemophilic patients, so that preventive and healthcare measures like more physical activity and consumption of vitamin D and calcium supplements could be provided. © 2018 Tehran University of Medical Sciences. All rights reserved.

Keywords: Bone structure-related biomarkers; Hemophilic patients

Introduction

The increased risk for developing loss of bone density in patients with hemophilia especially within childhood has been recently regarded (1). Losing bone density leading to osteoporosis has been reported in a wide range of hemophilic patients from 7.5 to 84 % (2,3). These pathological changes in bone structure may lead to arthropathy, severe osteoporosis, and even multifractures in later life. The exact mechanisms for osteoporosis secondary to hemophilia has not been fully elucidated, however, the main underlying reasons for lowering bone density in such condition include immobilization, lack of regular exercise, the elevation of bone-related turnover following secretion of pro-inflammatory cytokines, and recurrent hemarthrosis emphasizing the necessity for planning regular impact exercises in such patients (4). Chronic hepatitis C and HIV infections that are commonly occurred in hemophilic patients have been also found as other major causes for osteoporosis in hemophilia (5). Other risk factors for developing osteoporosis in hemophilia include small body size, poor diet, tobacco smoking, and high alcohol consumption (6). Along with immobility and lack of adequate physical activities, vitamin D deficiency has a fundamental role in increasing susceptibility of bone loss in hemophilic patients (7). In fact, because of pivotal role of vitamin D as a major mediator for bone mineralization, it is now hypothesized a critical role of hypovitaminosis in development of osteoporosis in hemophilic patients. In this regard, multidisciplinary approach including regular exercise programs, prophylactic replacement therapy, being warned of, and particularly optimizing calcium and vitamin D intake should be considered as the main strategies for osteoporosis in hemophilic patients (8).

According to the importance of assessing bone metabolism and its-related factors, all hemophilic
patients should be assessed regarding secondary causes of bone loss including the level of serum calcium for ruling out hyperparathyroidism or malnutrition, the level of 25-hydroxyvitamin D to assess vitamin D insufficient or sufficient, the level of alkaline phosphatase to rule out Paget disease, as well as liver and kidney functional biomarkers and enzymes to rule out hepatic or renal problems in these patients. The present study conducted to compare the levels of vitamin D and other biochemical factors affecting bone turnover in patients with hemophilia and those without this problem.

Materials and Methods

This case-control study was performed at Imam Khomeini general hospital in Tehran, Iran between 2014 and 2015. The study was to compare the level of bone-metabolism-related markers between the adult hemophilic patients and sex and age-matched healthy adults. In total, 57 patients who suffered hemophilia were included into the study. The definitive criteria for hemophilia included clinical manifestation, impaired PTT, and reduced levels of factor VIII or IX; the final diagnosis was definitively confirmed by the professional physician. All two groups were matched for age, sex, and socioeconomic level. According to the Italian guideline of diagnosis and treatment of osteoporosis, all participants were asked to take venous blood sample to assess the levels of serum biomarkers including total and free testosterone, thyroid stimulating hormone (TSH), Parathyroid hormone (PTH), vitamin D, creatinine (Cr), alkaline phosphatase (ALKp), calcium (Ca), phosphorus (P), osteocalcin, calcitonin, and bone biomarkers of cross-linked C-telopeptide (CTX) and cross-linked N-telopeptide (NTX). The levels of Ca, P, ALKp, and Cr were measured by spectrophotometry technique (Pars Azmoon Kit, Iran). The levels of vitamin D and TSH were assessed using the ELISA technique (Immunodiagnostic System LTD, UK) the level of lower than 10 ng/ml was considered as deficient, 10 to 29 ng/ml as insufficient, 30 to 100 ng/ml as sufficient, and higher than 100 as potential intoxication. The level of PTH was assessed using Colorimetric Immunoezymatic procedure (Diametra Kit, Italy). The serum total and free levels of testosterone and also calcitonin level was also measured using ELISA technique (Diametra Kit, Italy). Moreover, the levels of CTX and osteocalcin were assessed by ELISA technique (IDS kit, UK). The level of NTX was also measured using ELISA technique (Bioassay Technology Laboratory kit, China).

This study was undertaken after approval by the institutional ethical committee overseeing human studies. For statistical analysis, results were presented as the mean±standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using ANOVA test or Kruskal-Wallis H test whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. Categorical variables were, on the other hand, compared using chi-square test. P of ≤0.05 were considered statistically significant. For the statistical analysis, the statistical software SPSS version 23.0 for Windows (IBM, Armonk, New York) was used.

Results

In total, 57 hemophilic patients without any evidence of with HIV, HBV, or HCV infections were included as the case group, and 57 healthy individuals were considered as the controls.

<table>
<thead>
<tr>
<th>Index</th>
<th>Hemophilia without infection (n=57)</th>
<th>Non-hemophilic group (n=57)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free testosterone</td>
<td>10.90</td>
<td>10.63</td>
<td>0.005</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>2.69</td>
<td>2.85</td>
<td>0.012</td>
</tr>
<tr>
<td>TSH</td>
<td>2.83</td>
<td>3.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>12.62</td>
<td>38.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.17</td>
<td>1.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.39</td>
<td>9.58</td>
<td>0.036</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.53</td>
<td>3.54</td>
<td>0.257</td>
</tr>
<tr>
<td>ALKp</td>
<td>260</td>
<td>202</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CTX</td>
<td>260</td>
<td>230</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NTX</td>
<td>114</td>
<td>91</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>14.9</td>
<td>20.9</td>
<td>0.045</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>3.2</td>
<td>4.65</td>
<td>0.047</td>
</tr>
<tr>
<td>PTH</td>
<td>38.34</td>
<td>38.95</td>
<td>0.023</td>
</tr>
</tbody>
</table>

P of ≤0.05 were considered statistically significant*
Bone structure-related biomarkers

As shown in Table 1, comparing different biochemical markers related to bone metabolism and turnover showed significantly lower free testosterone, total testosterone, thyroid stimulating hormone (TSH), vitamin D, calcium, osteocalcin, calcitonin, and parathormone levels as well as higher serum alkaline phosphatase, CTX, and NTX levels in those hemophilic patients as compared to those without hemophilia.

Discussion

As clearly shown in the present study, hemophilia potentially induces some abnormal change in all aspects of bone metabolic pathways including bone calcium desorption, renal reabsorption of minerals as well as regulatory pathways related to bone structure and metabolism. In other words, regardless of sex and age, hemophilic patients are severely susceptible for bone defects due to affecting different structural and regulatory pathways related to bone metabolism and turnover and thus impact mineral and hormonal supporting bone structure in these patients. Previous studies show that osteoporosis is more frequent in hemophilic patients than the healthy population. In non-hemophilic population, women aged fewer than 35 are less than 1% likely to contract osteoporosis, well below 20% for women aged fewer than 60 (8,9). The association of hemophilia and abnormalities in bone structure and metabolism has been comprehensively studied in previous studies. As recently shown by Eldash et al., (9), vitamin D level, serum calcium, and bone mineral density were significantly lower in hemophiliacs than in control group that 43.2% of cases had moderate vitamin D deficiency and 35.1% had mild deficiency. Kempton et al., (10) indicated lower bone mineral density in hemophilic than the general population. In another study by Albayrak et al., (11), about 96% of hemophilic patients had low vitamin D levels. As similarly shown by Alioglu et al., (12), children with severe hemophilia could have significantly reduced bone mineral density, compared with sex- and age-matched healthy control subjects. Ranta et al., (13) also revealed that bone mineral density was lower in children with hemophilia, but there was no evidence for significantly increased fracture rate. The findings of the present study show that in hemophilic patients, there is risk for low bone density and subsequently osteoporosis. The patients had significantly higher urinary calcium excretion and higher serum calcium concentration, and reduced bone resorption as compared with the controls.

Similar to our observations, Avgeri et al., (14) showed that the hemophilic patients presented with higher levels in Glu-Oc, parathormone, and bone resorption markers, lower levels in bone formation markers and 25 (OH) D. In total, the pathophysiology of reduced bone mineral density and defected bone structure in hemophilic patients are not completely cleared, but it has been recently suggested that physical inactivity and vitamin D deficiency seem to play a fundamental role for such an association.

In different studies, osteoporosis has been recognized as an important factor of morbidity in hemophilic patients (15-16). It seems that the pathogenesis of Bone Mineral Density (BMD) reduction and the incidence of osteoporosis in hemophilic adults are most likely multi-factorial (17). The bone density is, in fact, a sort of balance between formation and resorption of bone. Any disruption in these two processes may cause osteoporosis (18,19). Osteoporosis is one of the important causes of disability and morbidity in hemophilic men and women as well as other blood-related complications (20). One of the factors causing bone mass loss and osteoporosis in hemophilia is liver malfunction due to a variety of reasons like viral infections, bleeding and inflammation, lack of physical exercise, and low weight (21). Each hemophilic patient must be examined individually for osteoporosis before prescribing a combination of medications and physical exercises (14). Simple methods of treatment include more physical activity, collective sport, prescription of sufficient vitamin D along with calcium and vitamin K, timely treatment of haemarthrosis, and primary prevention (14).

Hemophilic patients have lower BMI than they need for their age. An analysis (7) showed that hemophilic children and adults had significantly lower BMI than the healthy population.

Osteopenia and osteoporosis are among important health problems in hemophilic adults and children. The reasons of osteoporosis in these patients are diverse. It may result from insufficient physical exercise, frequent bleeding and inflammation, the presence of the inhibitor of a coagulation factor, low vitamin D, low BMI, severe disease and HCV, HBV or HIV infection. An appropriate screening protocol pertaining to osteoporosis must be implemented in the facilities for hemophilic patients so that preventive and healthcare measures like more physical activity and consumption of vitamin D and calcium supplements could start.
Acknowledgments

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References