The Roles of Osteoprotegerin and Rankl in Pathogenesis of Osteoporosis in Egyptian Beta Thalassemia Major Patients

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Abstract: Background: Two cytokines, osteoprotegerin (OPG) and receptor activator of nuclear factor kappa B ligand (RANKL), have recently been identified as important mediators in the pathogenesis of osteoporosis in patients with beta-thalassemia major (beta-TM). Aims: to characterize the possible role of the OPG –RANKL system in the pathogenesis of osteoporosis in beta-TM, as well as their relationship with bone turnover markers and bone mineral density (BMD). Subjects and Methods: In 39 Egyptian thalassemic patients and 30 healthy control subjects. Laboratory investigation included serum osteocalcin, carboxy-terminal propeptide of type I collagen(CICP), osteoprotegerin and RANKL and urinary deoxypyridinoline (DPD). Bone mineral density was measured by dual X-ray absorptiometry (DEXA) in spinal lumbar and femoral neck regions. Result: beta-TM patients showed an altered bone turnover, with an increased resorption phase [shown by significantly high levels of (DPD) (p=0.04)] and a decreased neoformation phase [shown by the low levels of osteocalcin and (CICP) (P=0.0001 for both )]. Moreover, they displayed significantly lower BMD values than controls both at the lumbar and femoral levels (P = 0.0001 for both). The thalassemic patients showed significantly lower serum levels of OPG (P=0.0001), whereas RANKL levels were significantly higher in beta-TM patients (P=0.001), who consequently showed a lower OPG/RANKL ratio (P=0.001) as compared with controls. Conclusion: Our data underline the important role played by the OPG –RANKL system in the development of osteoporosis in thalassemia major, which could be a potential target for novel therapeutic agents.

Key words: Thalassemia major, Osteoporosis, Osteoprotegerin, RANKL, Bone density, Bone turnover

INTRODUCTION

Beta-thalassemia major (beta-TM) represents a hemoglobinopathy caused by a hereditary defect in the synthesis of beta chain in adult hemoglobin, which results in ineffective erythropoiesis and increased peripheral hemolysis. Conventional management of beta-TM requires regular blood transfusions and adequate chelation therapy [1]. In recent years, advances in transfusion management and chelation therapy have achieved an improvement in skeletal development and cosmetic bone appearance. However, despite optimal conventional treatment and lack of endocrine complications, low bone density is still reported in thalassemic patients [2].

In fact, osteoporosis is an important cause of morbidity and disability in adult thalassemic patients presenting with different degrees of severity. The etiology of this bone disease is multifactorial (bone marrow expansion with cortical thinning, hypogonadotropic hypogonadism, hypothyroidism, hypoparathyroidism, diabetes mellitus, iron overload, deleterious effects of desferrioxamine on the bone metabolism, vitamin deficiencies, reduced physical activity and also genetic factors [3-5]. Many of these factors act by inducing an imbalance of bone remodeling, inhibiting the activation of the osteoblasts and / or stimulating osteoclasts function and thus culminates in a state of increased bone turnover with excessive bone resorption and remodeling.

Recently, the receptor activator of nuclear factor Kappa B(RANK), RANK-ligand (RANKL) and osteoprotegerin (OPG) has been recognized as an important mediator of osteoclastogenesis [6].

RANKL (soluble receptor activator of nuclear factor kB ligand), a member of tumor necrosis factor (TNF) superfamily, is produced by osteoblastic lineage cells and activated T cells and through the link with its receptor RANK on progenitor and mature osteoclasts, regulates development and activation of osteoclasts and bone remodeling.

OPG which is a member of the tumor
necrosis factor receptor (TNFR) superfamily is produced by a variety of cells and tissues (osteoblasts, lung, kidney, intestine, immune system cells) and acts as a decoy receptor for RANKL and blocks the RANKL-RANK interaction, inhibiting differentiation and activation of the osteoclasts and thus preserving the integrity of the bone. The RANK - RANKL and OPG balance is critical for osteoclastogenesis modulation and physiological bone remodeling.

The contribution of the OPG / RANKL system to the pathogenesis of bone disease in thalassemia has remained completely unknown. Thus in this study, to characterize the possible role of the OPG / RANKL system in bone loss related to thalassemia, we measured serum OPG and RANKL levels in 39 Egyptian β-TM patients and examined their potential relationship with the bone turnover markers and bone mineral density (BMD).

MATERIALS AND METHODS

A total of 39 patients with β-TM (16 girls and 23 boys, mean age 14.5±0.54 years, range 8-20 years) attending the hematology Clinic in Abou El Rich Hospital, Cairo University were enrolled into the study. In addition, 30 healthy subjects (16 girls and 14 boys, mean age 12.51±5.18 years, range 3-22 years) were used as a control group. Diagnosis of homozygous B-thalassemia was made using hemoglobin electrophoresis to identify variant hemoglobins. All patients were maintained on a regular transfusion program according to a monthly regimen with the aim of maintaining pretransfusional hemoglobin levels above 9 g/dl and iron chelating therapy (combination of desferrioxamine and deferiprone). Desferrioxamine administered subcutaneously by minipump at a dosage of 40 – 60 mg/kg/ day and was recommended to be used 5 nights /week. Oral chelator deferiprone administered at a dose of 75 mg/ kg/day in 2-3 divided doses and the dosage is adjusted according to serum ferritin level. None of the patients had any clinical or laboratory findings of endocrinopathy. All patients and healthy subjects gave informed consent form to participate in the present study, approved by National Research Centre Ethics Committee.

Bone Mineral Density: 28 patients and 30 controls were evaluated for reduced bone mineral density (BMD) of the lumber spine(L1-L4) and Lt femoral neck were performed at the Medical Services’ Centre, NRC. Absolute values were converted to Z-scores. Bone mineral density (BMD) was expressed in g/cm2 and BMC was expressed in gm.

Biochemical Analysis: Fasting blood samples were taken from all the participants for the measurement of the study parameters. Deoxypyridinoline (DPD) as a marker for osteoclastic activity & bone resorption, was measured in 2-h fasting morning urine sample by METRA DPD EIA kit (QUDEL Corp.).

Osteocalcin as a marker of osteoblastic activity & bone formation, was measured by host – ELIZA kit (Biosource Europe S. A), a quantitative sandwich Enzyme Linked Immuno-Sorbent Assay.

Carboxy – terminal propeptide of type I collagen (CICP) as a marker of bone formation, was measured in serum by METRA CICP EIA kit (QUDEL Corp.).

Osteoprotegerin (OPG) was measured in serum using ELIZA technique (Biovendor Laboratory Medicine, Inc., CzechRepublic for quantitative measurement.Cat. No RD 194003200) .

RANKL level was estimated by using ELIZA technique (Biomedica group, Biomedica Medicine product, GmbH & Co) for quantitative measurement of serum RANKL in EDTA plasma, heparin serum or cell culture supernatants, Cat. No. BI- 20422H.

Statistical Analysis: SPSS for Windows, version 10.0 computer program (SPSS, Chicago, IL., USA) was used for statistical analysis. A p value of less than 0.05 was considered statistically significant. The t-test was used to compare between 2 independent means. Pearson correlation coefficient “r” was used to measure the linear relationship between different continuous variables. Data are represented as the mean ± SD.

RESULTS

Biochemical and BMD data in β-TM patients and healthy controls are shown in Table 1. All biochemical data indicate an increase in the bone resorptive phase in β-TM subjects, as shown by significantly higher DPD levels (P=0.04) and decreased neoformation phase as shown by a significantly lower osteocalcin and CICP levels (P=0.0001 for both) as compared to controls.
Table 1: Biochemical and bone mineral density parameters in thalassemia major children and controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients (mean ± S.D)</th>
<th>Controls (mean ± S.D)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index “BMI” (kg/m²)</td>
<td>16.62±3.35</td>
<td>19.85±6.27</td>
<td>0.029*</td>
</tr>
<tr>
<td>Serum osteocalcin (mg/dl)</td>
<td>8.41±8.48</td>
<td>45±12.09</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Serum CICP (ng/ml)</td>
<td>274.86±255.4</td>
<td>470.62±203.62</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Urinary DPD (mmol/ mmol creatinin)</td>
<td>67.59±41.307</td>
<td>48.936±31.74</td>
<td>0.04*</td>
</tr>
<tr>
<td>Serum osteoprotegerin(OPG( ng/ml)</td>
<td>3.34±1.67</td>
<td>13.05±4.33</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Plasma RANK-L( ng/ml)</td>
<td>2.942±1.402</td>
<td>0.171±0.8</td>
<td>0.001*</td>
</tr>
<tr>
<td>Z score of femoral head</td>
<td>-0.8907±.569</td>
<td>-0.095±0.537</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Z score of lumbar region</td>
<td>-1.142±.66</td>
<td>-0.09±0.77</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

CICP: Carboxy – terminal propeptide of type I collagen.  
RANKL: receptor activator of nuclear factor kappa B.  
DPD: urinary deoxypyridinoline.  
*Denotes statistically significant value (P < 0.05)

Our DEXA results were categorized according to the following cut off points for children, the diagnosis of very low BMD (osteoporosis) defined as a BMD of 2 SD or more below the mean value (the Z score) compared to age- and sex-matched healthy controls. According to this approach, low BMD (osteopenia) would be said to exist when the BMD Z score lies between -1 and -2 SD [16]. Fig.1 shows the percentages of low BMD Z Scores in spinal and femoral neck regions. β-TM patients also showed significantly lower...
BMD values than controls, both at the lumbar and femoral levels (P=0.0001 for both). Fig.2 shows that the lumbar spine with its wide bone marrow spaces is more affected in our patients.

As shown in Table 1, OPG plasma levels were significantly lower in patients as compared with controls (P=0.0001), whereas RANKL plasma levels were significantly higher in patients (P=0.001), who consequently showed a lower OPG/RANKL ratio (P=0.001) as compared with controls. No statistically significant correlation was observed between the BMD Z-score and RANKL, OPG or OPG/RANKL ratio. There was also no correlation between the bone turnover markers and OPG and RANKL.

**DISCUSSION**

The life expectancy of patients with thalassemia has greatly improved over the last decade as a result of regular transfusions and increased compliance with iron chelation therapy, however, this improvement is often accompanied by a series of serious complications including osteopenia and osteoporosis. The pathogenesis of these skeletal disorders is multifactorial [17].

In the present study, β-TM patients showed significantly lower BMD values than controls, both at the lumbar and femoral levels.

In accordance with [18-21], our thalassemic patients displayed an unbalanced bone turnover with an increased resorption phase (shown by high urinary levels of DPD, a well known marker of bone resorption) and a decreased neoformation phase (shown by low levels of osteocalcin and CICP). These findings suggest that the decrement in bone density in thalassemia patients may be a consequence of uncoupling bone turnover.

With regard to the potential etiology for this unbalanced bone turnover, β-TM – related osteoporosis may develop because of the incessant marrow hyperplasia, secondary to the ineffective erythropoiesis [22]. This results in a dramatic expansion of bone marrow, almost 30-40 times more than normal, that has been considered as a major cause of bone destruction [23,24]. The intimate relation between bone marrow expansion and the process of remodeling is confirmed by the fact that β-TM – related bone loss largely involves the trabecular bone, in fact the lumbar spine with its wide bone marrow spaces is usually the most affected district in these patients [22].

However, our data suggest that the impact of medullar expansion on bone is not limited to mechanical destruction but it may also involve a paracrine influence of OPG / RANKL system which acts as an important paracrine mediator of bone metabolism in thalassemic patients.

The vast majority of the studies [4,18,19,20,25,26] indicates that an excessive osteoclastogenesis coupled to an inadequate osteoblastogenesis is responsible for the mismatch between bone formation and resorption in β-TM – related osteoporosis.

Since osteoclasts derive from bone marrow hematopoietic progenitors and the early stages of hematopoiesis and osteoclastogenesis proceed along similar pathways; [27] thus the hypothesis that hematopoietic chronic stimulation as determined by chronic anemia, may induce the proliferation of hematopoietic progenitor cells, thus increasing also the number of osteoclasts and accelerating bone loss, has recently received support [28].

The RANK – OPG system is recognized to be the main factor responsible for osteoclast activation. OPG protects bone from excessive resorption by binding to RANKL and preventing it from binding to RANK, thus the relative concentration of RANKL and OPG is a major determinant of bone mass and strength [29].

Voskaridou et al. [30] found a significant reduction in the OPG levels in a group of TM patients compared with the control group, while the RANKL levels varied widely within normal limits. In 30 osteoporotic patients with TM Morabito et al. [20] found a significant increase in RANKL compared to the control group, but no statistically significant difference in OPG levels between the groups, resulting in a significant decrease in the OPG / RANKL ratio. Voskaridou and Terpos [31] in 26 patients with TM and osteoporosis showed higher level of RANKL borderline significance respect to control and the mean OPG values were lower than controls; the OPG / RANKL ratio was significantly lower in thalassemia patients. Pietrapertosa et al. [32] unlike what has been reported in the literature, the serum levels of OPG were significantly higher in the thalassemic patients than in controls, the levels of RANKL were slightly higher in thalassemic patients, they were at the limit of significance compared with the control group; the OPG / RANKL ratio were higher in thalassemic than control group. In our study OPG was significantly lower and RANKL was significantly higher with a consequent reduction of the OPG / RANKL ratio which was significantly lower in thalassemics compared with the controls. This could represent the cause of uncoupling bone turnover observed in our patients. The multifactorial etiopathogenesis could be responsible for the heterogeneity of the results in the previous studies.
With regard to the relationship between OPG / RANKL system and BMD, data in the literature are not consistent. Our data, in accordance with Szulc et al. [32], Morabito et al. [20] and Pietrapertesa et al. [33] showed no correlation of OPG and RANKL serum concentrations with BMD at any site of measurement. These findings are in contrast with those of Yano et al. [34], who reported a negative correlation of OPG serum concentrations with BMD. In accordance with the data of Rogers et al. [35] and Morabito et al. [20], we found no significant relationships between OPG and urinary bone turnover markers. On this point, data in the literature are contrasting. In fact, whereas Szulc et al. [32] showed that serum OPG levels, in aging men. were negatively correlated with urinary DPD excretion, these findings do not support the data of Yano et al. [34], who reported positive correlations of serum OPG concentrations with the levels of biochemical markers of bone turnover (osteocalcin and DPD).

**Conclusions:** Reduced BMD is confirmed as an emerging problem in patients suffering from thalassemia major. Moreover, our data underline the important role played by the OPG – RANKL system in the development of osteoporosis in thalassemia major, which could be a potential target for novel therapeutic agents. Promising strategies include recombinant OPG and human monoclonal antibody to RANKL could help in reducing osteoclast activation and slow bone resorption so help in restoring a more physiological OPG/RANKL ratio.

**REFERENCES**

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