Thalassemic osteopathy: A new marker of bone deposition

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Abstract

Osteopathy represents a prominent cause of morbidity in patients with beta-thalassemia major (TM) and manifests as osteopenia/osteoporosis. Biochemical turnover markers (BTMs) are considered a useful, non-invasive tool for the clinical follow-up of osteoporotic patients; they can provide a dynamic view of the remodeling process and give information on the metabolic activity of bone tissue as well as on the pathogenesis of bone loss. The amino-terminal pro-peptide of type I procollagen (P1NP) is a recently introduced marker that is considered the most sensitive index of bone formation. Although demonstrated in several categories of patients with bone disease, there is little information on the clinical usefulness of this bone formation index in thalassemic patients. We evaluated the P1NP levels of 53 adult patients with thalassemia major (21 males and 32 females, mean age 46 years) and associated osteopathy. We investigated the correlation between P1NP and bone condition as examined by dual X-ray photon absorptiometry and with BTMs expressing bone resorption and bone mineralization (carboxyterminal collagen cross-linked (CTX) terminal regions of type I collagen and osteocalcin, respectively). P1NP serum levels were correlated with CTX levels \( r = 0.545, p < 0.001 \); the results were unchanged when males and females, as well as osteoporotic and osteopenic subgroups, were considered separately. No correlation was demonstrated neither between OC and CTX \( r = 0.17, p = \text{ns} \), nor between P1NP and OC levels \( r = 0.11, p = \text{ns} \); no correlation was demonstrated among the P1NP/CTX ratio and age, OC or densitometric values and no difference was found in the same ratio between osteopenic \( (0.19 ± 0.16) \) and osteoporotic \( (0.15 ± 0.14) \) patients. Similar results were obtained for the OC/CTX ratio, as it was not correlated with age, P1NP or densitometric values. This is the first report of circulating P1NP in patients with TM-associated osteoporosis. P1NP and CTX assays show good precision and low analytical CV, and, compared to other markers, they can acceptably reflect bone metabolic processes and promptly respond to antosteoporotic treatments. We trust that this sensitive marker can be useful in the assessment of treatment efficacy and can overcome the pitfalls due to wide variability in the normal values of most BTMs that create difficulty in pinpointing the individual patient’s response.

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is a marker of bone mineralization. Although these are undoubtedly valuable tools, the laboratory test results for both are influenced by technical aspects that impair sensitivity and increase variability, leading to reduction of clinical usefulness [5].

The amino-terminal pro-peptide of type I procollagen (P1NP) is a recently introduced marker that is considered the most sensitive index of bone formation in patients with bone disease of varying origins [6–12]. As information about circulating P1NP in patients with TM-associated osteoporosis is lacking, we evaluated the P1NP levels of a group of thalassemic patients with osteopenia or osteoporosis and investigated their correlation with other BTMs and bone conditions.

**Patients and methods**

**Patients**

From a large cohort of 150 adult patients with b-thalassemia major followed up at the Hereditary Anemia Center of Fondazione IRCCS Ca’ Granda Policlinico of Milan, we considered the data of 53 patients (21 males and 32 females, mean age 34.5 ± 5.7, range 22–46 years) with osteoporosis who underwent a complete bone evaluation for clinical purposes. All of the patients were regularly transfused and sufficiently chelated; furthermore, all were in a satisfactory control from the endocrinological point of view with treatment for hypogonadism, hypothyroidism and vitamin D deficiency.

**Methods**

Fasting blood samples for BTMs were obtained in the morning for all subjects via venipuncture and centrifuged within 1 h of blood sampling: the serum was frozen at −80 °C. Bone resorption was evaluated by measuring the carboxyterminal collagen cross-linked (CTX) terminal regions of type I collagen (CTX ELISA, IDS Nordin, Boldon Colliery, UK), which has normal values in post-menopausal women of 0.142–1.351 ng/mL, in pre-menopausal women of 0.112–0.738 ng/mL, and in males of 0.115–0.748 ng/mL. Bone formation was assessed by the serum levels of the N-terminal propeptide of type I procollagen or P1NP (UniQ P1NP, ORION Diagnostica, Espoo, Finland), which has normal values of 19–102 μg/L in females and 21–78 μg/L in males. Finally, bone mineralization was evaluated by assaying the levels of osteocalcin (N-MID Osteocalcin ELISA, IDS Nordin, Boldon Colliery, UK), which has normal values in post-menopausal women of 8.4–33.9 ng/mL, in pre-menopausal women of 12.8–55.0 ng/mL, and in males of 9.6–40.8 ng/mL. The assays were performed according to the manufacturer’s instructions, and a standard curve was included in each assay plate.

The bone densitometry scan was performed by dual X-ray photon absorptionmetry (Hologic Bone Densitometer QDR Discovery A, Version 12.7.3.1 WALTHAM, MA, USA) at the lumbar spine and femur in all of the patients; bone mineral density (BMD) was calculated as a ratio between bone mineral content (BMC) and area in square centimeters. The bone mineral density values were expressed as T- and Z-scores; the T-score was calculated as a standard deviation score (SDS) from a normal reference population database, while the Z-score was calculated as an SDS from an age- and sex-matched population. Data were classified according to the WHO report (WHO Technical Report, ISCD Official Position Paper 2007) as follows: T-score > −1 = normal, −1 > T-score > −2.5 = low bone density (osteopenia), T-score < −2.5 = osteoporosis.

**Statistical analysis**

Descriptive data (frequency, mean, standard deviation, median, range) were calculated for all variables; moreover, for P1NP variable percentiles also calculated. Pearson’s index was used to evaluate the correlations among CTX, OC, P1NP, bone mineralization, as expressed by T and Z-scores, and BTMs. Furthermore, partial correlation analysis was performed to evaluate the same correlations controlling for gender and bone mineralization status (osteopenia/osteoporosis). Finally, in consideration of the small size of the subgroups the nonparametric Mann–Whitney test was used to analyze the differences between genders and between hypogonadal/eugonadal patients. p values < 0.05 were considered significant. All statistics were calculated using the Statistical Package for the Social Sciences 20.0 for Windows software package (SPSS Inc., Chicago, IL).

**Results**

Among 53 TM patients, 41 (77%) were affected by osteoporosis and 12 (23%) by osteopenia. Eight patients (14.5%), 4 males and 4 females, were taking alendronate, which was prescribed in the presence of DXA values in the range for osteoporosis associated to high risk for fractures or previous fragility fractures.

The average values of BTM and bone mineral density are shown in Tables 1 and 2, respectively. There were 41 hypogonadal patients (77%), 17 males and 24 females, all on sex steroid replacement. There were no differences in the BTM levels neither between males and females nor between hypogonadal and eugonadal patients.

As shown in Fig. 1, P1NP serum levels were correlated with CTX levels (r = 0.54, p < 0.001); the results were unchanged when males and females, as well as osteoporotic and osteopenic subgroups, were considered separately. No correlation was demonstrated neither between OC and CTX (r = 0.17, p = ns), nor between P1NP and OC levels (r = 0.11, p = ns).

No correlation was demonstrated among the P1NP/CTX ratio and age, OC or densitometric values and no difference was found in the same ratio between osteopenic (0.19 ± 0.16) and osteoporotic (0.15 ± 0.14) patients. Similar results were obtained for the OC/CTX ratio, as it was not correlated with age, P1NP or densitometric values.

**Discussion**

There is great interest in the etiology and management of thalassemic osteopathy due to the high prevalence of this complication even in well-treated thalassemic patients. The determination of biochemical markers of bone turnover provides important information about bone metabolism disorders and treatment responses; thus, their use should be implemented. Among the greatest advantages of these markers are their non-invasiveness, repeatability and ability to change quickly in response to treatment. Although recent laboratory improvements have increased the ability of BTM assays to characterize bone disease, both biological variability and laboratory variability compromise their usefulness in reflecting bone metabolism [3,5,8,13,14]. The expert opinion is that no perfect marker exists [5].

Initially, the total alkaline phosphatase in serum and urinary hydroxyproline were adopted as bone turnover indices, but they were later replaced by bone-specific alkaline phosphatase and OC. The former
reflects the cellular activity of osteoblasts, while the latter is considered a formation marker whose exact function remains unclear [5]. Unfortunately, the high within-method interlaboratory variability (CV 16–48% and 16–42%, respectively) [15] and major drawbacks of these assays impose considerable limitations on their clinical application. In particular, the reliability of bone alkaline phosphatase is impaired by cross-reactivity between bone and liver isozymes. However, the rapid degradation of OC in serum and incorporation into the bone matrix lead to the degradation of the native peptide in heterogeneous fragments, which can be detected by commercial kits even with poorly defined specificity and cross-reactivity [5]. Furthermore, OC is greatly influenced by genetics and, though related to fracture risk, is not considered a responsive indicator of bone metabolism changes [16,17].

Recently, the serum concentrations of some byproducts of collagen and proteins released during osteoid deposition were shown to reflect the bone formation rate [18,19]. Among these byproducts, the aminoterminal propeptide (P1NP) of type I procollagen, which represents 95% of total bone collagen, is considered to possess the highest specificity and sensitivity. Furthermore, as its serum concentrations are stoichiometrically equivalent to the new collagen molecules synthesized by osteoblasts [20], P1NP is considered to most accurately reflect changes in new collagen synthesis [6]. In addition, although none of the collagen formation byproducts are specific for bone, it is believed that most P1NP is produced during bone deposition.

Although demonstrated in several categories of patients with bone disease [7,9] there is little information on the clinical usefulness of this bone formation index in thalassemic patients; though recognizing the limits of the present study due to the low number of patients and the wide range of results, we believe that our experience can be considered an interesting first step to be continued with a larger study. A further limit of the present study is the cross-sectional design; the absence of correlation between BMTs and bone mineral density can be explained as bone metabolism is a dynamic multifactorial process, and a longitudinal study could better demonstrate the relationships between the changes in bone metabolism and the resulting mineral density.

We did not find significant differences between hypogonadic and eugonadic thalassemic pts neither for bone turnover markers nor for DXA parameters expressing bone mineralization. We observed, however, an interesting trend to higher levels in the two markers of bone degradation (P1NP and osteocalcin) in the hypogonadal group, where the serum concentrations are stoichiometrically equivalent to the new collagen molecules synthesized by osteoblasts [20]. P1NP is considered to most accurately reflect changes in new collagen synthesis [6]. In addition, although none of the collagen formation byproducts are specific for bone, it is believed that most P1NP is produced during bone deposition.

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![Fig. 1. Correlation between amino-terminal pro-peptide of type I procollagen (P1NP) and carboxy-terminal cross-linking telopeptide of type I procollagen (CTX) serum levels in the patients with thalassemia major.](image-url)
planning a prospective study including a P1NP assay in TM before and after anti-osteoporotic treatment. We trust that this sensitive marker can reveal useful in the assessment of treatment efficacy and can overcome the pitfalls due to wide variability in the normal values of most BTMs, that create difficulty in pinpointing the individual patient’s response.

Conclusion

P1NP and CTX are biochemical bone turnover markers widely used for monitoring patients with primary and secondary osteoporosis, as they can acceptably reflect bone metabolic processes and promptly respond to anti-osteoporotic treatments, and the assay methods are provided with good precision and low analytical CV [21,22]. To the best of our knowledge, this is the first report of P1NP serum levels in patients with TM-associated osteoporosis; the measurement of this sensitive marker in a larger series of TM could be useful in the clinical follow-up of the changes in bone metabolism related to disease and drug response. Further studies on this topic are anticipated with great interest, as they will help in the decision-making process.

Conflict of interest

None of the authors had actual nor potential conflict of interest.

References