Evaluation of Hormonal Influence in Patients with Fractures Attributed to Osteoporosis*

Avaliação da influência hormonal em pacientes com fraturas atribuídas a osteoporose

Danila Malheiros-Souza[1] Leonardo Franco Pinheiro Gaia[1] Fausto Fernandes de Almeida Sousa[1] Pedro Ivo Ferreira Favaro[1] Virmondes Rodrigues[1] Denise Bertulucci Rocha Rodrigues[1,2]

[1] Departament of Biological Sciences, Immunology Laboratory, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil
[2] Immunobiology Laboratory, Universidade de Uberaba, Uberaba, MG, Brazil

Address for correspondence Denise Bertulucci Rocha Rodrigues, PhD, Universidade de Uberaba, Uberaba, MG, Av Nenê Sabino, 1801, Uberaba MG, 38055-500, Brazil (e-mail: denise.rodrigues@uniube.br).

Rev Bras Ortop 2021;56(6):804–808.

Abstract

Objective The present study aims to evaluate the influence of hormonal levels of vitamin D, calcitonin, testosterone, estradiol, and parathyroid in patients with fractures attributed to osteoporosis when compared with young patients with fractures resulting from high-impact accidents.

Methods Blood samples were collected from 30 elderly patients with osteoporosis-attributed fractures (T-score $\leq$ -2.5) (osteoporotic group), and from 30 young patients with fractures resulting from high-impact accidents (control group). Measurement of 1,25-hydroxyvitamin D (Kit Diasorin, Saluggia, Italy), calcitonin (Kit Siemens, Tarrytown, NY, USA), testosterone, estradiol, and parathyroid hormone (Kit Beckman Couter, Indianapolis, IN, United States) was performed using a chemiluminescence technique. Data were inserted into a Microsoft Excel (Microsoft Corp., Armonk, WA, USA) spreadsheet and analyzed using Statview statistical software. Results showing non-normal distribution were analyzed with nonparametric methods. The Mann-Whitney test was applied for group comparison, and a Spearman test correlated hormonal levels. Statistical significance was set at $p < 0.05$. All analyzes compared gender and subjects with and without osteoporosis.

Results Women with osteoporosis had significantly lower levels of estradiol and vitamin D ($p = 0.047$ and $p = 0.0275$, respectively). Men with osteoporosis presented significantly higher levels of parathyroid hormone ($p = 0.0065$). There was no significant difference in testosterone and calcitonin levels.

Keywords
- osteoporosis
- hormones
- estradiol
- calcitonin
- parathyroid hormone

* *Study developed at the Immunology Laboratory, Department of Biological Sciences, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil.*

received
August 5, 2020
accepted
December 1, 2020
published online
August 27, 2021

DOI https://doi.org/10.1055/s-0041-1726065.
ISSN 0102-3616.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

Conclusion Osteoporosis patients presented gender-related hormonal differences. Women had significantly lower levels of estradiol and vitamin D, whereas men had significantly higher parathyroid hormone levels, apparently impacting the disease.

Resumo

Objetivo Avaliar a influência dos níveis hormonais de vitamina D, calcitonina, testosterona, estradiol e paratormônio em pacientes com fratura atribuída a osteoporose, quando comparados com pacientes jovens que tiveram fraturas decorrentes de acidente de alto impacto.

Métodos Foram coletadas amostras de sangue de 30 pacientes idosos com fratura atribuída a osteoporose (T-score $\leq$ -2,5) (grupo com osteoporose) e 30 amostras de sangue de pacientes jovens que sofreram fraturas decorrentes de acidentes de alto impacto (grupo controle). Foram realizadas dosagem de 1,25-hidroxivitamina D (Kit Diasorin, Saluggia, Italy), calcitonina (Kit Siemens, Tarrytown, NY, USA), testosterona, estradiol e paratormônio (Kit Beckman Couter, Indianapolis, IN, United States) pela técnica de quimiluminescência. Os dados foram inseridos em uma planilha de dados no programa Microsoft Excel (Microsoft Corp., Redmond, WA, EUA) e analisados pelo programa de estatística Statview. Os resultados que apresentaram distribuição não normal foram analisados com métodos não paramétricos. Para análise de variáveis comparando-se os dois grupos, aplicou-se o teste Mann-Whitney. Foi utilizado o teste de correlação de Spearman para a correlacionar os níveis hormonais. Um valor-p $>$0.05 foi considerado significante. Todas as análises foram feitas comparando gênero e grupos de pacientes com e sem osteoporose.

Resultados Mulheres com osteoporose apresentam níveis significativamente menores de estradiol e vitamina D ($p = 0.047$ e $p = 0.0275$), respectivamente. Homens com osteoporose demonstraram níveis significativamente maiores de paratormônio ($p = 0.0065$). Não houve diferença significativa nos níveis de testosterona e calcitonina.

Conclusão Existem diferenças hormonais entre os gêneros na osteoporose. Em mulheres, níveis significativamente menores de estradiol e vitamina D e, nos homens, níveis significativamente maiores de paratormônio, parecem influenciar na doença.

Palavras-chave
- osteoporose
- hormônios
- estradiol
- calcitonina
- hormônio paratireóideo

Introduction

Osteoporosis results from an imbalance in normal bone metabolism between osteoblasts and osteoclasts.[1] Osteoclasts seem more active than osteoblasts in bone resorption processes.[2,3] Studies indicate that hormones may play an important role in imbalanced bone formation,[4] since parathyroid hormone apparently induces osteocytes differentiation into osteoclasts.[5] Likewise, hormones such as estradiol and testosterone are supposedly important,[6–8] and testosterone acts by inhibiting osteoblast apoptosis.[9] On the other hand, estrogen seems critical to bone remodeling both in males and females, since it apparently stimulates calcitonin release and activates intestinal vitamin D receptors, resulting in endocrine and immune functions during bone metabolism.[10,11] Calcitonin and vitamin D help to maintain adequate serum concentrations of calcium to allow normal bone mineralization. In addition, vitamin D is required for bone growth and remodeling by osteoblasts and osteoclasts.[12]

The increase in life expectancy resulted in a considerable increase in diseases associated with hormonal changes, including osteoporosis, warranting the focus on studies to clarify these interactions.

The present study aims to evaluate the influence of hormonal serum levels of vitamin D, calcitonin, testosterone, estradiol, and parathyroid hormone in patients with fractures attributed to osteoporosis in comparison with young patients with fractures resulting from high-impact accidents.

Materials and Methods

The present project was approved by the Ethics Committee under protocol number 51827515.4.0000.5145.

Study Group

Blood samples were collected from 30 elderly patients with fractures attributed to osteoporosis (T-score $\leq$ -2.5) (osteoporotic group), and from 30 young patients with fractures resulting from high-impact accidents (control group). Patients with other bone conditions, nonosteoporotic fractures, immunosuppression, malignant neoplasms, or liver disorders were excluded from the study, along with those who did not agree to participate in it. Serum collected from these patients was used for hormone measurement.

Blood Collection

Venous blood collection was always performed in the morning, 1 day after the bone reconstruction surgery indicated by the orthopedist. A blood sample was obtained by venipuncture, using three vacuum collection tubes containing a clot activator and separating gel. Thirty minutes after collection, the samples were centrifuged at 5,000 rotations per minute (rpm) for 10 minutes to obtain serum.

Hormonal Analysis

Serum obtained after blood samples centrifuging was sent for hormonal analysis. Measurement of 1,25-hydroxyvitamin D (Kit Diasorin, Saluggia, Italy), calcitonin (Kit Siemens, Tarrytown, NY, USA), testosterone, estradiol, and parathyroid hormone (Kit Beckman Couter, Indianapolis, IN, United States) was performed using a chemiluminescence technique, a chemical reaction that generates luminous energy. Chemiluminescence reagents are transformed into electrically excited intermediate states and release the absorbed energy as light when becoming less excited.

Statistical Analysis

Data were inserted into a Microsoft Excel (Microsoft Corp., Redmond, WA, USA) spreadsheet and analyzed using Statview statistical software. Results showing non-normal distribution were analyzed with nonparametric methods. The Mann-Whitney test was applied for group comparison, and a Spearman test correlated hormonal levels. Statistical significance was set at $p < 0.05$.

Results

The present study measured hormones in 30 patients with osteoporosis and in 30 control subjects, totaling 60 people.

The mean age of the patients was 58.8 ± 22.61 years old, and all analyzes compared gender and subjects with or without osteoporosis. ►**Table 1** shows the number of patients and the mean age from each group.

In the control group, serum vitamin D levels were significantly higher in females when compared with males (Mann-Whitney test; $p = 0.0169$). Comparing both groups (osteoporosis and control subjects), serum vitamin D levels were significantly higher in women from the control group than in women with osteoporosis ($p = 0.0275$). There was no significant difference between males and females from the osteoporosis group (►**Figure 1A**).

Significantly higher free testosterone levels were observed in males when compared with females in both the control and osteoporotic groups (Mann-Whitney test; $^{*}p = 0.0023$ and $^{**}p = 0.0046$). There was no significant difference in free testosterone levels between males from the control and osteoporotic groups (►**Figure 1B**).

Estradiol levels were significantly lower in women with osteoporosis compared with those of the control group (Mann-Whitney test; $p = 0.047$). There was no significant difference in estradiol levels between men and women from the control group and the osteoporotic group (►**Figure 1C**).

Parathyroid hormone levels were significantly higher in men with osteoporosis when compared with the control group (Mann-Whitney test; $p = 0.0065$). There was no significant difference between women from the control group and women with osteoporosis. No significant difference was observed in parathyroid hormone levels when comparing males and females with or without osteoporosis (►**Figure 1D**).

There was no significant difference in calcitonin levels between the control and osteoporotic groups, regardless of gender. In addition, there was no significant difference when comparing men and women from both groups (►**Figure 1E**).

Discussion

Osteoporosis results from an imbalance of bone remodeling potentially caused by hormonal factors. In addition, more recent studies show that immunological factors also play a role in the pathophysiology of the disease. The present study evaluated hormonal levels in patients with osteoporosis and compared gender-related differences.

Our study revealed significant differences in vitamin D, estradiol, and parathyroid hormone levels. Osteoporotic women and young men had significantly lower levels of vitamin D compared with young women. Studies show that vitamin D deficiency is associated with muscle weakness, bone loss, falls and fractures.[13] In line with the literature, our data suggest that a decrease in vitamin D in older women may contribute to osteoporosis. The present study also showed that reduced estradiol levels are related to the onset of osteoporosis in women > 60 years old, which is consistent with other works, showing that bone health is inversely related to lower estradiol

Table 1 Mean, minimum, and maximum age (in absolute numbers) in female and male subjects with and without osteoporosis

	Mean Age (years old)	Number of Patients (*n*)	Minimal Age (years old)	Maximal Age (years old)
Female control subjects	39.5	08	18	58
Male control subjects	39.6	22	19	58
Women with osteoporosis	80.05	18	64	98
Men with osteoporosis	74.9	12	60	88
Total	58.8	60	18	98

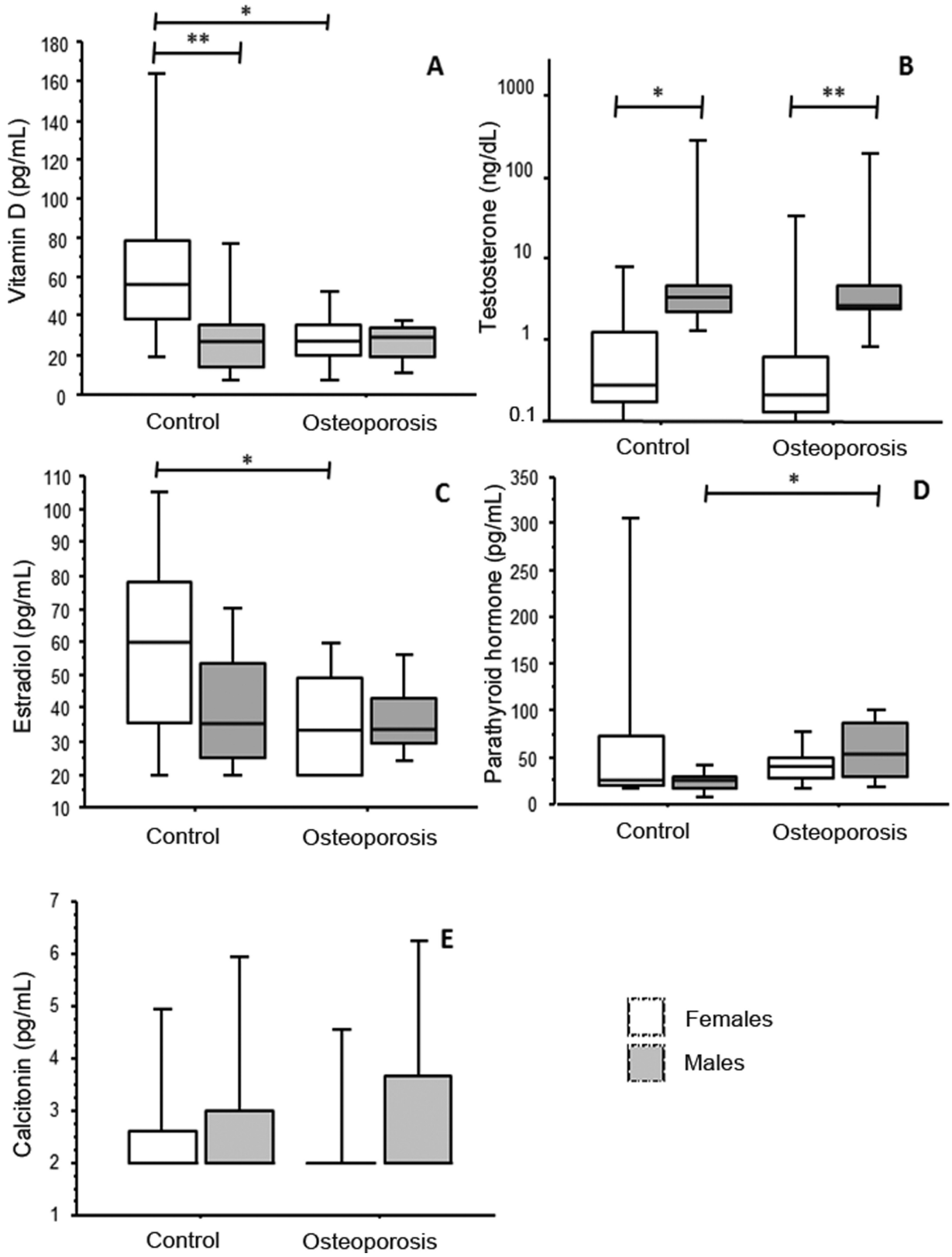

Fig. 1 Hormonal measurement using chemiluminescence in female and male patients diagnosed with osteoporosis and control subjects. (**A**) Vitamin D, $^{*}p = 0.0169$ and $^{**}p = 0.0275$; (**B**) Testosterone, $^{*}p = 0.0023$ and $^{**}p = 0.0046$; (**C**) Estradiol, $p = 0.047$; (**D**) Parathyroid hormone, $p = 0.0065$; (**E**) Calcitonin (Mann-Whitney test).

concentrations.[7,8,14] Estradiol deficiency was also linked to osteoporosis in men > 64 years old,[15] although this association was not observed here.

Bone remodeling is also stimulated by parathyroid hormone. A study with elderly women showed a significant increase in parathyroid hormone levels in women with osteoporosis.[16] In the present study, significantly higher levels of parathyroid hormone were found in men with osteoporosis, showing that it contributes to the onset of the disease in men > 60 years old; in contrast, women present no differences in parathyroid hormone levels.

Testosterone was not a limiting factor for the onset of osteoporosis in our patients. This hormone seems to be more related to differences between males and females. However, a study showed that testosterone deficiency in men > 64 years old is associated with rapid bone loss, leading to osteoporosis.[15] Even though the literature showed that calcitonin acts by inhibiting bone resorption,[17] we found no significant differences in calcitonin levels between groups and genders.

Conclusion

The present study suggests that women with osteoporosis had significantly lower levels of estradiol and vitamin D compared with young women without the disease, whereas men with osteoporosis had significantly higher levels of parathyroid hormone compared with men without the disease. These findings show the importance of hormones and vitamin D in the development of osteoporosis. Although lower testosterone levels are associated with osteoporosis, our study does not show its impact on the disease, except for a significant gender-related difference, regardless of age and of the presence of osteoporosis.

Authors contributions

All authors contributed to the conception and design of the study. The study was designed by Rodrigues V. e Rodrigues D. B. R. Gaia, L. F. P., Sousa F. F. A., Favaro, P. I. and Malheiros-Souza D. collected the material, prepared the database, and performed the statistical analyzes. Malheiros-Souza D. and Rodrigues D. B. R. wrote the manuscript and revised it. Rodrigues V. revised the manuscript.

Funding Sources

The present study was supported by the Universidade de Uberaba, the Universidade Federal do Triângulo Mineiro, and the Centro de Educação Profissional, in addition to having received financial support from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, in the Portuguese acronym), from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, in the Portuguese acronym), and from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, in the Portuguese acronym).

Conflict of Interests

The have no conflict of interests to declare.

References

1 Zaidi M. Skeletal remodeling in health and disease. Nat Med 2007; 13(07):791–801
2 Charles JF, Coury F, Sulyanto R, et al. The collection of NFATc1-dependent transcripts in the osteoclast includes numerous genes non-essential to physiologic bone resorption. Bone 2012;51(05): 902–912
3 Udagawa N, Takahashi N, Yasuda H, et al. Osteoprotegerin produced by osteoblasts is an important regulator in osteoclast development and function. Endocrinology 2000;141(09):3478–3484
4 Breckon JJ, Papaioannou S, Kon LW, et al. Stromelysin (MMP-3) synthesis is up-regulated in estrogen-deficient mouse osteoblasts in vivo and in vitro. J Bone Miner Res 1999;14(11):1880–1890
5 Silva BC, Bilezikian JP. Parathyroid hormone: anabolic and catabolic actions on the skeleton. Curr Opin Pharmacol 2015; 22:41–50
6 Damien E, Price JS, Lanyon LE. Mechanical strain stimulates osteoblast proliferation through the estrogen receptor in males as well as females. J Bone Miner Res 2000;15(11):2169–2177
7 Gui Y, Duan Z, Qiu X, et al. Multifarious effects of 17-β-estradiol on apolipoprotein E receptors gene expression during osteoblast differentiation in vitro. Biosci Trends 2016;10(01):54–66
8 Kousteni S, Han L, Chen JR, et al. Kinase-mediated regulation of common transcription factors accounts for the bone-protective effects of sex steroids. J Clin Invest 2003;111(11):1651–1664
9 Wiren KM, Toombs AR, Semirale AA, Zhang X. Osteoblast and osteocyte apoptosis associated with androgen action in bone: requirement of increased Bax/Bcl-2 ratio. Bone 2006;38(05): 637–651
10 Adams JS. Vitamin D as a defensin. J Musculoskelet Neuronal Interact 2006;6(04):344–346
11 Chen H, Gilbert LC, Lu X, et al. A new regulator of osteoclastogenesis: estrogen response element-binding protein in bone. J Bone Miner Res 2011;26(10):2537–2547
12 Weaver CM, Alexander DD, Boushey CJ, et al. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National Osteoporosis Foundation. Osteoporos Int 2016;27(01):367–376
13 Bischoff-Ferrari HA, Conzelmann M, Dick W, Theiler R, Stähelin HB. [Effect of vitamin D on muscle strength and relevance in regard to osteoporosis prevention]. Z Rheumatol 2003;62(06):518–521
14 Kousteni S, Bellido T, Plotkin LI, et al. Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 2001;104(05):719–730
15 Fink HA, Ewing SK, Ensrud KE, et al. Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. J Clin Endocrinol Metab 2006;91(10):3908–3915
16 Al-Daghri NM, Aziz I, Yakout S, et al. Inflammation as a contributing factor among postmenopausal Saudi women with osteoporosis. Medicine (Baltimore) 2017;96(04):e5780
17 Martin TJ, Sims NA. Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med 2005;11(02): 76–81