Unbalanced bone remodeling in Tunisian patients with inflammatory bowel diseases

Sameh Hadj Taieb[1], Lamia Kallel[2], Moncef Feki[1], Mohamed B. Hammami[1], Jalel Boubaker[2], Mohamed Hsairi[3], Azza Filali[2], Naziha Kaabachi[1]

[1]*Tunis El Manar University, Faculty of Medicine of Tunis, Laboratory of Biochemistry, Tunis, Tunisia*
[2]*Tunis El Manar University, Faculty of Medicine of Tunis, Department of Gastroenterology, Tunis, Tunisia*
[3]*Tunis El Manar University, Faculty of Medicine of Tunis, National Institute of Health, Tunis, Tunisia*

S. Hadj Taieb, L. Kallel, M. Feki, M. B. Hammami, J. Boubaker, M. Hsairi, A. Filali, N. Kaabachi

Déséquilibre du remodelage osseux chez des patients Tunisiens atteints de maladies inflammatoires chroniques de l'intestin

LA TUNISIE MEDICALE - 2013 ; Vol 91 (n°04) : 273-277

S. Hadj Taieb, L. Kallel, M. Feki, M. B. Hammami, J. Boubaker, M. Hsairi, A. Filali, N. Kaabachi

Unbalanced bone remodeling in Tunisian patients with inflammatory bowel diseases

LA TUNISIE MEDICALE - 2013 ; Vol 91 (n°04) : 273-277

R É S U M É

Prérequis : La perte osseuse est une complication souvent ignorée au cours des maladies inflammatoires chroniques de l'intestin. Ses mécanismes ne sont pas totalement élucidés.
But : Etudier le remodelage osseux chez les patients atteints de maladies inflammatoires chroniques de l'intestin.
Méthodes: Les produits de dégradation urinaire des télopeptides C-terminaux du collagène de type I, l'ostéocalcine sérique, la parathormone, la 25 hydroxy-vitamine D et l'interleukine-6 ont été dosés chez 67 patients atteints de maladies inflammatoires chroniques de l'intestin et 54 sujets sains appariés selon l'âge et le sexe. La densité minérale osseuse a été mesurée par l'absorptiométrie biphotonique aux rayons X et l'ostéoporose a été définie par un T score <-2,5 DS.
Résultats: Les patients ont montré des taux significativement plus élevés des télopeptides C-terminaux du collagène de type I et d'interleukine-6, et plus bas de 25 hydroxy vitamine D. L'ostéocalcine et la parathormone étaient normaux. En analyse multivariée, les télopeptides C-terminaux du collagène de type I étaient associés à l'activité de la maladie (p = 0,04) et l'ostéocalcine a été associée à la parathormone (p = 0,04). Les télopeptides C-terminaux du collagène de type I et l'interleukine-6 étaient significativement augmentés chez les patients avec ostéoporose. Absence d'association entre l'ostéoporose et l'ostéocalcine sérique, la parathormone et la 25 hydroxy vitamine D.
Conclusion: La résorption osseuse est augmentée et est associée à l'ostéoporose chez les patients atteints de maladies inflammatoires chroniques de l'intestin. L'inflammation, la malnutrition et l'hypovitaminose D peuvent contribuer à cette perte osseuse.

M o t s - c l é s

Remodelage osseux, maladies inflammatoires chroniques de l'intestin, ostéoporose, hypovitaminose D

S U M M A R Y

Background: Bone loss is an ignored complication in inflammatory bowel diseases. Its underling mechanisms are not fully elucidated.
Objectives: To investigate bone turnover in patients with inflammatory bowel diseases.
Methods: The study included 67 patients with inflammatory bowel diseases and 54 age- and sex-matched healthy subjects. Urinary degradation products of C-terminal telopeptide of type I collagen, serum osteocalcin, parathyroid hormone, 25 hydroxy vitamin D and interleukin-6 were assessed. Bone mineral density was measured by dual energy-X-ray absorptiometry and osteoporosis was defined as T score < -2.5 SD.
Results: Patients showed significantly higher levels of C-terminal telopeptide of type I collagen and interleukin-6 and lower levels of 25 hydroxy vitamin D. Serum osteocalcin and parathyroid hormone were in normal range. In multivariate analysis, urinary degradation products of C-terminal telopeptide of type I collagen were associated with disease activity (p=0.04) and osteocalcin was associated with parathyroid hormone (p=0.04). Urinary degradation products of C-terminal telopeptide of type I collagen and interleukin-6 were significantly increased in inflammatory bowel disease patients with osteoporosis. No association was found between osteoporosis and serum osteocalcin, parathyroid hormone and 25 hydroxy vitamin D.
Conclusion: Bone resorption rate is increased and is associated with osteoporosis in inflammatory bowel disease patients. Inflammation, malnutrition, and hypovitaminosis D may contribute to the bone loss.

K e y - w o r d s

Bone remodeling, inflammatory bowel disease, osteoporosis, hypovitaminosis D

Osteopenia and osteoporosis are common extra intestinal complications in inflammatory bowel disease that increase the risk of disabling vertebral crushes and femoral neck fractures [1-4]. Bone tissue is constantly being renewed and repaired by a coupled process of resorption and formation called bone remodeling. The rate of bone remodeling can be assessed by the measurement of bone matrix compounds or bone derived enzymes, released into the circulation during bone formation and resorption [5, 6]. Some characteristics of inflammatory bowel disease, such as malabsorption of calcium and vitamin D, increased inflammatory cytokines and use of steroids are known to have negative effect on bone metabolism [7-12]. Previous studies investigating bone metabolism in inflammatory bowel disease provided conflicting results on the rate of bone remodeling and the role of the factors involved in bone metabolism regulation [2, 13-19]. This study was aimed to estimate the rate of bone remodeling in inflammatory bowel disease patients through the measure of urinary degradation products of C-terminal telopeptide of type I collagen as marker of bone resorption and serum osteocalcin as marker of bone formation, and to test their association with bone regulatory factors such as parathyroid hormone, vitamin D and interleukin-6, as well as some clinical and radiological parameters.

MATERIAL AND METHODS

Subjects

Sixty-seven patients presenting with IBD [44 with Crohn's disease (CD) and 23 with ulcerative colitis (UC) patients] at Gastroenterology A department of Rabta hospital (Tunis) were included. Fifty four age- and sex-matched healthy subjects from Rabta hospital employees and their relatives were elected as controls. The diagnosis of CD and UC had been previously established on the basis of clinical, endoscopic and histological criteria. Disease activity was evaluated through Truelove and Wittes criteria for UC [20] and Best index for CD [21]. Patients with UC limited to the rectum and patients with spondylarthropathy, renal failure, primary sclerosing cholangitis, dysthyroidism or pregnancy were excluded. For each patient, the following data were collected: age, gender, disease duration from diagnosis to inclusion (expressed in months), intestinal localization of CD, and extent of colonic involvement for UC, history of surgical resection, cumulative steroid dose (expressed in g prednisone equivalent), body mass index (BMI) in Kg/m^2 and menopausal status for females. No patient had received bone medication (bisphosphonates or others). The protocol of the study was approved by the ethics committee of Rabta Hospital (Tunis, Tunisia), and all participants gave their informed consent to participate to the study.

Methods

Biochemical markers

Blood and urine (second morning void) samples were collected from fasted individuals and aliquots of serum and urine were stored at -80°C until analysis (within 3 months). Urinary CrossLaps were measured with a micro plate competitive ELISA method using polyclonal antibodies reactive with the amino acid sequence of EKAHD-β-GGR, where the aspartic acid residue (D) is β-isomerised (Osteometer Biotech; normal range: premenopausal female, 0.4-9.15 mg/g creatinine; menopausal female, 0.3-8.77 mg/g creatinine; male, 0.43-4.09 mg/g creatinine). Serum osteocalcin was measured with a human specific two site radioimmunometric assay that recognizes the large N-terminal mid fragment in addition to the intact molecule (ELSA-OSTEO Cis Biointernational; normal range, women 8-56 μg/L; men 5.2-37 μg/L). Serum intact parathyroid hormone was measured by a two-site radioimmunometric assay (Cis Biointernational; normal range, 11-62 ng/L). 25 hydroxy vitamin D (25 OHD) was determined by a competitive radioimmunoassay method after extraction with acetonitrile (Diasorin; normal range, 10-45 μg/L). Serum interleukin-6 (IL-6) was measured by a micro plate sandwich ELISA method using 2 monoclonal anti-IL-6 antibodies (Immunotech; normal range, 1-16 ng/L).

Bone mineral density

Bone mineral density (BMD) of the lumbar spine and left femoral neck was measured in IBD patients by dual energy-X-ray absorptiometry (Sophos XRA, version VX 1-3). Results were expressed as T score. Patients with T score < -2.5 standard deviation (SD) in spine or femoral neck were considered osteoporotic [22].

Statistical analysis

Statistical analysis was carried out using SPSS for windows 10.0. Software (SPSS Inc.). Differences between groups were compared by Student t test for continuous variables and by Chi-squared tests for categorical variables. Correlations between continuous variables were assessed using Spearman rank correlation. Multiple and logistic regression models were applied to determine independent factors associated with markers of bone resorption (CrossLaps) and formation (osteocalcin), and with osteoporosis in IBD patients. Goodness-of-fit of logistic models were satisfactory. P value <0.05 was considered significant.

RESULTS

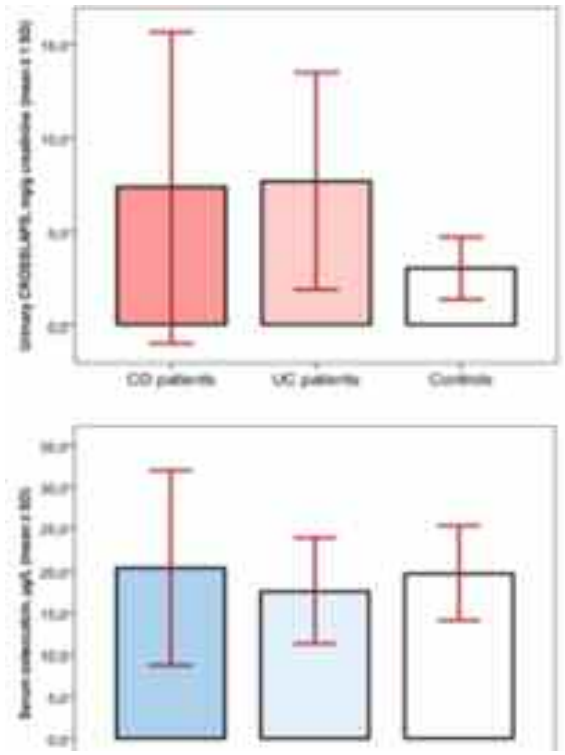
The main characteristics of study subjects were showed in table 1. Both CD and UC patients showed significantly higher CrossLaps and Il-6 levels and lower 25 OHD means levels in comparison with controls. Whereas, osteocalcin and intact parathyroid hormone were in normal ranges in both patients groups (figure 1, table 2). IBD patients with active disease had significantly higher CrossLaps (8.5 ± 6.1 vs 5.7 ± 3.0 mg/g creatinine; p=0.02) (figure 2) and IL-6 (24.7 ± 12.9 vs 15.9 ± 7.2 ng/l; p=0.001) concentrations than those in remission. There was a trend meaningful increase of CrossLaps levels in menopausal women (9.1 ± 7.3 vs 6.7 ± 4.2 mg/g creatinine; p=0.09). No significant differences were found for all these markers regarding to type of IBD, gender and previous intestinal resection.

Table 1: Main clinical characteristics of patients according to type of inflammatory bowel disease

	Crohn's disease patients (n=44)	Ulcerative colitis patients (n=23)
Male gender, %	54.5	47.8
Current smokers, %	25.6	13.0
Age, years	34.1 ± 11.0	43.1 ± 14.7
Body mass index, kg/m²	20.5 ± 4.7	23.5 ± 5.1
Disease course, months	74.5 ± 62.6	57.7 ± 48.9
Cumulated dose of corticoids*	5.52 ± 6.52	2.56 ± 5.81
Active disease, %	47.7	56.5
Previous surgical resection, %	27.9	8.72
Menopausal women, %	30.0	28.6

* , g equivalent of prednisone

Figure 1: Comparative distribution of urinary CrossLaps and serum osteocalcin concentrations in patients with Crohn's disease (CD) and patients with ulcerative colitis (UC) with controls



In univariate analysis, CrossLaps were positively related to osteocalcin (r=0.27; p=0.04), and inversely related to BMI (r= -0.23; p<0.05), and BMD in spine (r= -0.30; p<0.05) and in femoral neck (r= -0.24; p<0.05). Osteocalcin was related to PTH (r=0.31; p=0.02). No correlation was observed between CrossLaps or osteocalcin levels and age, disease duration, cumulated dose of steroids, and plasma PTH, 25 OHD and IL-6 concentrations. After adjusting on several potential confounding factors (i.e. age, gender, smoking status, type of IBD, disease duration, disease activity, cumulated dose of steroids, PTH, 25 OHD and IL-6), CrossLaps were found to be associated with disease activity (p=0.04) and osteocalcin with PTH (p=0.04).

IBD patients with osteoporosis showed significantly higher CrossLaps (table3) and IL-6 levels. But, no difference of osteocalcin, PTH and 25 OHD levels was observed between IBD patients with or without osteoporosis (table 3). When applying a binary logistic regression modeling including age, gender, BMI, smoking status, type of IBD, disease activity, disease duration, previous intestinal resection and cumulated dose of steroids) as confounding variables, osteoporosis was found to be associated with BMI [Odd-ratio (OR), 95% confidence interval (95% CI), 0.86 (0.74-0.98); p= 0.03)] and active disease [OR (95% CI), 3.30 (1.01-11.1); p= 0.04].

Figure 2 : Urinary CrossLaps concentrations (mg/g creatinine) by disease activity (active disease versus quiescent disease)

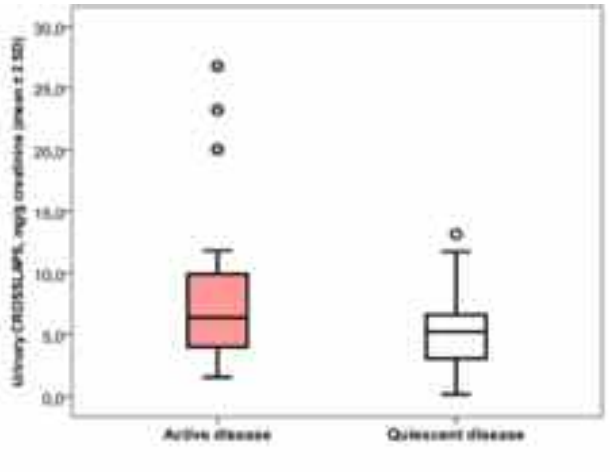


Table 2: Biochemical markers of bone metabolism in patients with inflammatory bowel disease and controls

	Patients			Controls (n=54)
	IBD patients (n=67)	CD patients (n=44)	UC patients (n=23)	
CrossLaps, mg/g creatinine	7.11 ± 4.92**	7.62 ± 5.43**	6.82 ± 4.71**	3.01 ± 1.72
Osteocalcin, µg/L	19.5 ± 9.95	18.1 ± 6.11	20.4 ± 11.63	17.1 ± 5.92
Parathyroid hormone, ng/L	30.3 ± 13.1	33.0 ± 10.8	28.8 ± 14.2	30.3 ± 8.7
25 hydroxy vitamin D, µg/L	9.4 ± 11.4**	10.1 ± 9.5**	8.9 ± 9.2**	22.7 ± 9.9
Interleukin-6, ng/L	20.4 ± 11.4**	18.2 ± 8.3**	21.6 ± 12.6**	9.0 ± 4.9

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; * p<0.05; ** p<0.001 (in comparison with controls)

Table 3: Biochemical markers of bone metabolism in inflammatory bowel disease patients according to osteoporosis

	Osteoporosis (T score < -2.5)	
	No (n=42)	**Yes (n=25)**
CrossLaps, mg/g creatinine	6.01 ± 4.31	9.24 ± 5.52*
Osteocalcin, μg/L	18.7 ± 8.52	20.8 ± 12.0
Parathyroid hormone, ng/L	31.7 ± 14.1	28.0 ± 10.2
25 hydroxy vitamin D, μg/L	9.11 ± 8.33	9.86 ± 10.7
Interleukin-6, ng/L	19.5 ± 12.4	21.9 ± 9.40

*, P < 0.05

DISCUSSION

Characterization of bone turnover is useful as previous data showed that biochemical markers of bone metabolism may help to estimate the risk of rapid bone loss, to choose and monitor therapeutic intervention, and to predict long-term osteodensitometric response to therapy [5, 23, 24]. Previous studies using biochemical bone markers in IBD have produced conflicting results, showing either increased bone resorption without a compensatory increase in bone formation [2, 13, 17, 19, 25], decreased bone formation with no variation in resorptive markers [14, 15, 18], decreased [19] or increased [16, 26-28] both bone formation and resorption markers. The present study showed significantly higher CrossLaps levels in patients with IBD than healthy controls. Moreover, CrossLaps were correlated with BMD and were increased in IBD patients with osteoporosis. These findings suggest that activation of bone resorption is an important mechanism of bone loss in IBD patients. Accordingly, the prospective study by Dresner-Pollak and al [2] showed that urinary N-telopeptide crosslinked type 1 collagen (NTx), another bone resorption marker, was inversely correlated with spinal BMD rate of change. The authors found that patients with the highest NTx quartile experienced the greatest decrease in spine BMD compared with patients with the lowest NTx quartile. Thus, regular assessment of these urinary markers may be useful in predicting bone loss in IBD patients. The pathogenesis of osteopenia and osteoporosis in IBD has been suggested to result from pathological rates of bone turnover arising from multi factorial mechanisms such as deleterious effects of circulating cytokines and mediators released by the inflamed intestines, prolonged corticosteroid therapy, sex hormone deficiency, reduced physical activity and bowel resection [29]. However, studies investigating mechanisms of bone loss in IBD provided conflicting results on the role of age, gender, type of IBD, disease duration, disease activity, cumulated dose of steroids, smoking, malabsorption, malnutrition, and inflammation [1, 14-19]. In these patients, biochemical markers of bone remodeling were not related to age, gender, smoking status, duration of disease and the type of IBD, whereas CrossLaps were significantly increased in patients with active disease. This increase was associated with elevated IL-6 levels suggesting a key role of inflammation in enhancing bone resorption. Indeed, IL-6 and TNF-α present at high concentrations in inflamed bowel and plasma of patients with active disease [30], appear to influence bone remodeling often resulting in bone loss [10]. In this study, serum IL-6 was increased in IBD patients especially those with active disease, but was not associated with markers of bone turnover and BMD. This suggests that other cytokines such as IL-1 and TNF-α may influence bone metabolism or that serum IL-6 do not reflect the role of this cytokine in the microenvironment of the bone tissue. Recent studies [31, 32] offered a new perspective on the pathogenesis of bone loss in IBD, made possible by one of the most important advancements in bone biology; the discovery of the OPG/RANKL/RANK system, the main activating system of osteoclasts [33]. Moschen et al [31] found a negative correlation between plasma osteoprotegerin (OPG) levels and spine and femoral neck BMD and suggested that the increased levels of OPG could be a «homeostatic response, attempting to reverse established osteopenia and RANKL-driven osteoclastogenesis».

This study showed an independent inverse relationship between osteoporosis and BMI, suggesting a key role of malnutrition in bone loss in IBD patients. In fact, intestinal absorption is frequently altered and calcium and vitamin D are among the poorly absorbed substances [7, 34, 35]. Vitamin D deficiency and disturbances of calcium metabolism would be a factor contributing to bone loss in IBD [12]. A recent review on vitamin D status in IBD reported hypovitaminosis D in up to 65% of adult patients [36]. Several reasons have been suggested for the low vitamin D status in patients with IBD including reduced intestinal absorption of vitamin D as a consequence of ileopathy, disrupted entero-hepatic circulation of this vitamin, renal insufficiency and reduced dietary intake and exposure to sunshine [28]. In this study, 25 OHD levels were low in IBD patients, but were associated neither with markers of bone turnover nor with osteoporosis. Moreover, hypovitaminosis D was not associated with increased PTH concentrations. These results are in accordance with those of Robinson [37] and Vogelsang [38]. Thus, we speculate that calcium is released from bone through PTH-independent mechanisms, such as inflammatory cytokines and corticosteroid therapy. Corticosteroids are recognized to have profound effects on bone metabolism [39]. However, the relationship between corticosteroids and osteoporosis in IBD remains controversial [1, 9, 11, 13, 19, 40]. In this study, cumulated dose of corticosteroids was not associated with bone remodeling markers or osteoporosis.

CONCLUSION

In total, bone metabolism is unbalanced in IBD patients with increased bone resorption but no evident variation of bone formation. Elevated CrossLaps was found to be associated with active disease and low BMI, suggesting a key role of inflammation and malnutrition in bone loss in IBD patients. Optimal prevention and treatment of osteoporosis in IBD remains to be established. However, anti-resorptive agents could be tested in IBD patients with an elevated resorption rate.

Références

1. Robinson R J, Al Azzawi F, Iqbal S J et al. Osteoporosis and determinants of bone density in patients with Crohn's disease. Dig Dis Sci 1998;4:2500-6.
2. Dresner-Pollak R, Karmeli F, Eliakim R, Ackerman Z, Rachmilewitz D. Increased urinary N-telopeptide cross-linked type I collagen predicts bone loss in patients with inflammatory bowel disease. Am J Gastroenterol 2000;95:699-704.
3. Reinshagen M. Osteoporosis in inflammatory bowel disease. J Crohn's Colitis 2008;2:202-7.
4. Bianchi ML. Inflammatory bowel diseases, celiac disease, and bone. Arch Biochem Biophys 2010;503:54-65.
5. Eastell R. The value of biochemical markers of bone turnover in osteoporosis. J Rheumatol 1997;24:1215-7.
6. Briot K, Roux C. Biochemical markers of bone remodeling. Gynecol Obstet Fertil 2005; 33:1009-13.
7. Walters JRF. The role of the intestine in bone homeostasis. Eur J Gastroenterol Hepatol 2003;15:845–9.
8. Hylemon P.B, Zhou H, Pandak W.M, Ren S, Gil G, Dent P. Bile acids as regulatory molecules. J Lipid Res 2009;50:1509–20.
9. De Nijs RN. Glucocorticoid-induced osteoporosis: a review on pathophysiology and treatment. Minerva Med 2008;99:23-43.
10. Tilg H, Moschen AR, Kaser A, Pines A, Dotan I. Iinflammation and osteoporosis: basic and clinical concepts. Gut 2008;57:684-94.
11. Bernstein CN, Seeger LL, Sayre JW, Anton PA, Artinian L, Shanahan F. Decreased bone density in inflammatory bowel disease is related to corticosteroid use and not disease diagnosis. J Bone Miner Res 1995;10:250-6.
12. Jahnsen J, Falch JA, Mowinckel P, Aadland E. Vitamin D status, parathyroid hormone and bone mineral density in patients with inflammatory bowel disease. Scand J Gastroenterol 2002;37:192-9.
13. Silvennoinen JA. Karttunen TJ, Niemelä SE, Manelius JJ, Lehtola JK. A controlled study of bone mineral density in patients with inflammatory bowel disease. Gut1995;37:71-6.
14. Bischoff SC, Herrmann A, Göke M, Manns MP, von zur Mühlen A, Brabant G. Altered bone metabolism in inflammatory bowel disease. Am J Gastroenterol 1997;92:1157-63.
15. Abitbol V, Roux C, Chaussade S et al. Metabolic bone assessment in patients with inflammatory bowel disease. Gastroenterology 1995;108:417-22.
16. Pollak RD, Karmeli F, Eliakim R, Acherman Z, Tabb K, Rachmilewitz D. Femoral neck osteopenia in patients with IBD. Am J Gastroenterol 1998;93:1483-90.
17. Bjarnason I, Macpherson A, Mackintosh C, Buxton-Thomas M, Forgacs I, Moniz C. Reduced bone density in patients with inflammatory bowel disease. Gut 1997;40:228-33.
18. Schoon EJ, Geerling BG, Van Dooren IM et al. Abnormal bone turnover in long-standing Crohn's disease in remission. Aliment Pharmacol Ther 2001;15:783-92.
19. Schulte C, Dignass A, Mann K, Goebell H. Reduced bone mineral density and imbalanced bone Metabolism in patients with inflammatory Bowel disease. Inflamm Bowel Dis 1998;4:268-75.
20. Truelove SC, Witts LJ. Cortisone in ulcerative colitis. Br Med J 1955;2:1041-6.
21. Best WR, Becktel JM, Singleton, Kern F. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology 1976;70:439-44.
22. Kanis JA, Melton LJ 3rd, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. J Bone Miner Res 1994;9:1137-41.
23. Cashman KD. Altered bone metabolism in inflammatory disease: role for nutrition. Proc Nutr Soc 2008;67:196-205.
24. Watts NB. Clinical utility of biochemical markers of bone remodeling. Clin Chem 1999;45:1359-68.
25. Gilman J, Shanahan F, Cashman KD. Altered levels of biochemical indices of bone turnover and bone-related vitamins in patients with Crohn's disease and ulcerative colitis. Aliment Pharmacol Ther 2006;23:1007-16.
26. Ardizzone S, Bollani S, Bettica P, Bevilacqua M, Molteni P, Bianchi Porro G. Altered bone metabolism in inflammatory bowel disease: there is a difference between Crohn's disease and ulcerative colitis. J Intern Med 2000;247:63–70.
27. Miheller P, Toth M, Molnar E, Zágoni T, Rácz K, Tulassay Z. Serum bone marker measurements in bone metabolism disorders associated with inflammatory bowel diseases. Orv Hetil 2001;142:1557-60.
28. McCarthy D, Duggan P, O'Brien M et al. Seasonality of vitamin D status and bone turnover in patients with Crohn's disease. Aliment Pharmacol Ther 2005;21:1073-83.
29. Trebble TM, Wootton SA, Stroud MA et al. Laboratory markers predict bone loss in Crohn's disease: relationship to blood mononuclear cell function and nutritional status. Aliment Pharmacol Ther 2004;19:1063-71.
30. Hyams JS, Fitzgerald JE, Treen WR, Wyzga N, Kreutzer DL. Relationship of functional and antigenic interleukin-6 to disease activity in inflammatory bowel disease. Gastroenterology 1993;104:1285-92.
31. Moschen AR, Kaser A, Enrich B et al. The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 2005;54:479-87.
32. Bardella MT, Bianchi ML, Teti A. Chronic inflammatory intestinal diseases and bone loss. Gut 2005;54:1508.
33. Khosla S. Minireview: The OPG/RANKL/RANK system. Endocrinology 2001;142:5050-5.
34. Bano G, Rodin DA, Pazianas M, Nussey SS. Reduced bone mineral density after surgical treatment for obesity. Int J Obes Relat Metab Disord 1999;23:361-5.
35. Di Stefano M, Veneto G, Malservisi S, Corazza GR. Small intestine bacterial overgrowth and metabolic bone disease. Dig Dis Sci 2001;46:1077-82.
36. Pappa HM, Grand RJ, Gordon CM. Report on the vitamin D status of adult and pediatric patients with inflammatory bowel disease and its significance for bone health and disease. Inflamm Bowel Dis 2006;12:1162-74.
37. Robinson RJ, Iqbal SJ, Abrams K, Al-Azzawi F, Mayberry JF. Increased bone resorption in patients with Crohn's disease. Aliment Pharmacol Ther 1998;12:699-705.
38. Vogelsang H, Ferenci P, Woloszczuk W et al. Bone disease in vitamin D-deficient patients with Crohn's disease. Dig Dis Sci 1989;34:1094-9.
39. Sambrouk PN, Jones G. Corticosteroid osteoporosis. Br J Rheumatol1995;34:8-12.
40. Ledro Cano D, Torres Domínguez Y, Castro Laria L, Sáenz Dana M, Herrerías Esteban JM, Herrerías Gutiérrez JM. Biochemical markers and bone densitometry in inflammatory bowel disease. Rev Esp Enferm Dig 2000;92:595-600.