International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 04-05-2019; Accepted: 06-06-2019
www.medicalsciencejournal.com
Volume 5; Issue 7; July 2019; Page No. 20-24

Vitamin D, Osteocalcin, C-terminal telopeptide mature Type 1 collagen (CTX) in the development of medication-related osteonecrosis of the jaws

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Abstract
Prospective study of patients with medically related osteonecrosis of the jaw admitted to the Maxillofacial Surgery Clinic, St. Marina University Hospital, Varna University was conducted in 24 patients with bisphosphonate treatment with (13 pts) and without(11 pts) osteonecrosis of the jaw, biomarkers of bone metabolism vitamin D, osteocalcin, C-terminal telopeptide mature Type I collagen (CTX) were studied. There were low vitamin D levels, low normal levels of osteocalcin and C-terminal telopeptide of mature type I collagen (CTX). There were no statistical differences between MRONJ + and MRONJ-, but the odds ratio showed an increased risk of medically related osteonecrosis of the jaw 3.2 higher with osteocalcin levels below 7.8 ng / ml and 1.92 times higher with CTX values below 0.150 ng / ml.

Keywords: Medically related osteonecrosis of the jaw (MRONJ), biomarkers, vitamin D, osteocalcin, C-terminal telopeptide mature Type 1 collagen (CTX)

Introduction

Medically related osteonecrosis of the jaws (MRONJ) is a side effect of long-term treatment with bisphosphonates and lately with other antiresorptive drugs such as RANK-L inhibitors (Denosumab) and the diagnosis is being based on persistent oral lesions of necrotic mandibular or maxillary bone that can cause pain or be asymptomatic [1, 2, 3, 4]. Over the past 10 years this has become a growing problem associated with increased frequency of osteonecrosis and the addition of new groups of drugs like antiangiogenenic agents being involved. Osteonecrosis of the jaws is a rare but potentially serious side effect of this treatment of malignant tumors and metastatic bone disease or myelomatosis patients on intravenous administration protocols and now it is also a problem for many patients with osteoporosis on oral bisphosphonate protocols [5, 6, 7, 8, 9]. In 2014. The American Association of Oral and Maxillofacial Surgery (AAOMS) recommends the term medication-associated osteonecrosis of the jaws (MRONCH) and assumed that jaw bone osteonecrosis may be present if the following criteria are met: (a) current or prior treatment with anti-resorptive or anti-angiogenic agents; (b) exposure of bone that can be probed through an intraoral or extraoral fistula in the maxillofacial area lasting for more than eight weeks; c) no evidence of previous radiotherapy in the area [1, 2].

Bone elasticity and strength, collagen protein and calcium mineral component are of particular importance in the pathophysiology of MRONJ. The bone matrix is a two-phase system in which the mineral phase provides strength and the collagen fibers provide elasticity and energy absorption capacity Changes in collagen metabolism may affect the bone's mechanical properties and increase fracture risks. Type I collagen is the most common type of collagen and is widespread in almost all connective tissues. It is the major protein in bones and skin and accounts for 95% of bone collagen and about 80% of bone protein [10, 11, 12]. Decreased bone collagen production, as shown by the bone resorption markers, is invisible in bone mineral density (BMD) examination using X-ray osteometry. On the other hand, the prognostic ability of the BMD for fracture risk assessment does not exceed 50% multiple clinical trials since only the mineral phase, rather than the overall bone structure, is evaluated [27, 28, 29].

In experimental studies decreased viability of oral keratinocytes was also found, which could be the likely explanation for bone exposure in the clinical presentation of MRONJ [21, 22]. Prolonged administration of bisphosphonates may result in bone microfractures visualized by a scanning electron microscope, due to the elevated chewing pressure in both lower and upper jaw seen in both animal models and clinical trials. The presence of microfractures is associated with reduced bone remodeling through suppressed osteoclast function as well as a first asymptomatic phase of the disease which, after an initiating factor such as tooth extraction and bacteria microfilm insertion may progress to its second and manifest phase of pain, bone sequestration and inflammation [23, 24].

Vitamin D deficiency is widespread especially in the elderly and is considered a risk factor for vascular, neoplastic and

neurodegenerative diseases. The status of vitamin D has been investigated in patients with MRONJ and studies have been conducted to increase vitamin D levels in such patients.

Vitamin K act as a coenzymes for vitamin K-dependent carboxylase enzyme that catalyzes the carboxylation and activation of glutamic acid (GLA). This carboxylating reaction is essential for the formation of bone collagen which allows the bone to deform upon impact, during chewing or falling Vitamin K-dependent gamma-carboxylation occurs only on specific glutamic acid residues in a small number of proteins, but is essential for the calcium-binding function of these proteins and proper calcium deposition in bones and not in arterial walls [30-34].

Osteocalcin is a protein that is synthesized by osteoblasts and is regulated by the active form of vitamin D. The calcium binding capacity of osteocalcin requires vitamin K-dependent gamma-carboxylation of 3 residues of glutamic acid. Increased non-carboxyllated osteocalcin (ucOC) is associated with an increased risk of fractures and the use of vitamin K in clinical trials resulted in a decrease in non-carboxyllated osteocalcin and tendency to reduce fracture risk. Serum levels of osteocalcin reflect the intensity of bone turnover, in particular bone formation, with particularly high levels observed in bone mineral disease. Activated matrix Gla protein (MGP) is another vitamin K-dependent protein that blockes calcium deposition in arterial walls and slowing calcification of vessels in the presence of vitamin K [35 38].

The etiology of MRONJ has been extensively studied, but consensus on basic pathophysiology has not yet been reached [21-24]. The hypotheses on MRONJ etiology include: a) suppressed angiogenesis and presence of immunosuppression leading to avascular necrosis [20], (b) suppression of bone remodeling due to reduced viability of osteoclasts and osteocytes and osteoblasts; c) suppression of collagen exchange in bones and soft tissues with decreased viability of oral keratinocytes [21]; fibroblast suppression [22] and the appearance of microfractures [23, 24]; d) infection with oral microbial biofilms migrating to exposed jaw bone after dental procedures [20, 21] and (e) the role of vitamin D and vitamin K in bone remodeling and osteonecrosis pathogenesis [25-26, 30, 31].

Materials and methods

Prospective study of patients with MRONJ admitted to the Department of Oral and Maxillofacial Surgery and Special Image Diagnostics, Faculty of Dental Medicine, St. Marina University Hospital, Varna University, Bulgaria. In 24 patients with bisphosphonate treatment 13 with osteonecrosis and 11 without osteonecrosis of the jaws vitamin D concentrations and biomarkers osteocalcin and C-terminal telopeptide of mature collagen type I (CTX) were studied.

Laboratory Methods

Vitamin D - determined by ECLIA - an immunological, electrochemical, high-value method. An automated analyst Elecsys 2010, Roche Diagnostics, Switzerland, was used to determine the total volume 25 (OH). Its levels depend on parathyroid hormone and calcitonin. Normal values above 30 ng / ml (50 nmol / L) (Serious deficiency <12 ng / ml (25 nmol / L), Deficiency 12-30 ng / ml).

Osteocalcin - The Liason® Osteocalcin Test of Dia Sorin, MN, USA is a one-step immunohistochemistry method designed to work with a Liason Analyzer series immunological analyzer with two incubations involving murine antibodies to human osteocalcin, photoluminescence with calibration curve results. Osteocalcin is the major non-collagenous protein of the bone matrix, constituting 1-3% of the protein content of the bone and is synthesized by osteoblasts. Osteocalcin is a protein molecule containing 3 amino acid residues of gamma-carboxyglutamic acid (GLA), vitamin K-dependent, and its synthesis is regulated by calcitriol. Normal values - menopausal women 5.4-59.1 ng / ml; men 4.6 - 65.4 ng / ml.

C-terminal collagen type I telopeptide (CTX), B-Cross Laps (β-CTX) - Investigated by the electrochemiluminescence method of the ECLIA test designed to work with the Elecsys Immunoassay Analyzer and quantified for the collagen I cleavage products containing 2 molecules of isomeric octapeptide 8βAA is performed on a sandwich principle with two incubations with a test duration of 18 minutes and is read by a calibration curve. Normal values 0.035-0.950 ng / ml.

Statistical methods

Statistical data grouping - the variables are sorted according to their type in variation, interval, category statistic rows. Statistical estimation method - point estimates - for arithmetic mean of continuous variables and interval estimates for statistical significance - p, mean statistical deviation (SD). Independent t-test (Students-Fisher) to compute and discriminate continuous variables and correlation coeficients.

Graphic methods - Microsoft Office Professional Plus Exel is used to visualize and describe demographic data and patient risk characteristics.

Odds ratio OR) by the formula OR = ad / b.ca = Number of exposed cases b = Number of exposed non-cases c = Number of non-exposed cases) d = Number of unexposed non-cases (number of non-participant cases)

Results

Biomarkers of bone metabolism in patients with and without MRONJ

Table1 shows the values of vitamin D, osteocalcin and C-terminal telopeptide of collagen type 1(CTX).

In both groups with and without MRONJ, vitamin D values are low in all patients. There were no statistically significant differences in the values of all three biomarkers when comparing MRONJ+ to MRONJ- groups. There is an odds ratio at which the risk of developing of MRONJ is 3.2 higher when osteocalcin levels arebelow 7.8 ng/ml and 1.93 higher when C-terminal collagen type I telopeptides are below 0.150 ng/ml.

Table 1: Values of vitamin D, osteocalcin, and CTX in patients with MSDN and non-MSD.

Patients	Number	Age	Vitamin D ng/ml Serious deficit < 12 Deficit 12 – 30 Normal > 30	Osteocalcin ng/ml Females menopause 5,4-59,1 Males4.6 - 65.4	C-terminal telopeptide mature Type 1 collagen (CTX) ng/ml 0.035-0.950ng/ml
MRONJ+	13	64±11	18,2±11,5	11,5±7,8	0,13±0,075

MRONJ -	11	63±8,2	11,92±6.6	10,3±4,8	0,18±0,17
p			0,06	0,67	0,31
Odds ratio				3,2(when <7,8 ng/ml)	1,92(when<0,150 ng/ml)

In Table 2 the distribution of values of vitamin D, osteocalcin and CTX values are shown.
Vitamin D values are severely deficient in 67% of MRONJ+ and in 82% of MRONJ - patients. Osteocalcin deficiency is found in 38% of MRONJ + and in 9% of MRONJ -patients. CTX values are below normal in 9% of MRONJ+ and in 0% of MRONJ- patients, but all concentrations are at the lowest quartile of the reference values. (Table 3, Figure 8)

Table 2: Distribution of patients according to the values of vitamin D, osteocalcin and CTX

Biomarker	Values	MRONJ+%	MRONJ -%
Vit D	<12 ng/ml	67	82
	>12<30 ng/ml	33	18
	>30 ng/ml	0	0
Ost	<5.4(4.6♂) ng/ml	38	9
	>5.4(4.6♂) ng/ml	62	91
CTX	<0.035ng/ml	9	0
	>0.035ng/ml	91	100

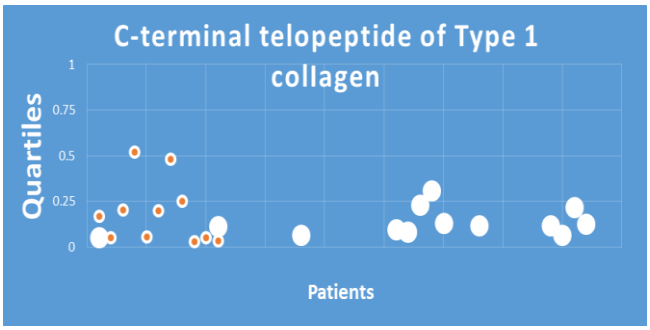

Fig 1: Distribution according to C-terminal telopeptide mature Type 1 collagen (CTX)) ng/ml in both MRONJ+(white dots) and MRONJ%- patients predominantly in the lowest quartile of the reference values.

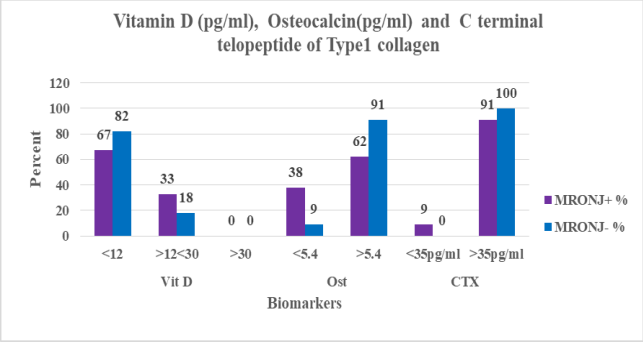

Fig 2: Values of vitamin D, osteocalcin and CTX in patients with MRONJ+and MRONJ.

Discussion

The etiology of MRONJ has been extensively studied, but consensus on basic pathophysiology has not yet been reached [21-24]. The hypotheses on MRONJ etiology include: a) suppressed angiogenesis and presence of immunosuppression leading to avascular necrosis [20]; (b) suppression of bone remodeling due to reduced viability of osteoclasts and osteocytes and osteoblasts; c) suppression of collagen exchange in bones and soft tissues with decreased viability of oral keratinocytes [21]; fibroblast suppression [22] and the appearance of microfractures [23, 24]; d) infection with oral microbial biofilms migrating to exposed jaw bone after dental procedures [20, 21] and (e) the role of vitamin D and vitamin K in bone remodeling and osteonecrosis pathogenesis [25-26, 30, 31].
The role of vitamin D in patients with MRONJ is not well-established and it is generally suggested that low vitamin D levels may predispose to the development of MRONJ. In a 2-year retrospective study vitamin D values were compared in patients with unexposed to exposed bone and a significant difference between groups was found (29.5 vs. 20.49 ng / ml) with the recommendation to adequate substitute vitamin D in patients treated with antiresorptive drugs. [27] In another study biomarkers of bisphosphonate treated MRONJ + and MRONJ - patients were compared and there was no differences in vitamin D, parathyroid hormone, bone alkaline phosphatase, C terminal collagen telopeptide I values. Vitamin D was low (defined as 25-OH-D <50 nmol / l) in 59 % of MRONJ + and 62% of MRONJ – patients (p = 0.77) so it was suggested that vitamin D deficiency is not a risk factor for MRONJ [29].Vitamin D levels in this study were low and we found no differences in vitamin D levels between both groups of bisphosphonate treated and MRONJ positive patients.
Intravenous bisphosphonate treatment reduces C-terminal telopeptide mature Type 1 collagen (CTX) by 50% and this reduction is considered a biomarker confirmation of pharmacological effect of bisphosphonates. Since the hypothesis of bisphosphonate suppression of collagen turnover in bones and soft tissues with decreased viability of oral keratinocytes and fibroblasts [21, 22] collagen biomarkers received greater attention in the context of medically related necrosis of jaws and oral mucosa. It was demonstrated that upon discontinuation of bisphosphonate C-terminal telopeptide mature Type 1 collagen (CTX) and osteocalcin showed a significant increase over time (p = 0.007). Bone remodeling was studied in a study of MRONJ + and MRONJ – patients treated with bisphosphonates and CTX levels of less than 150 pg / mL were associated with an elevated odds ratio of development of MRONJ of 5.268 (P =. 004). C-terminal telopeptide (CTX) level of less than 150 pg / mL was suggested as a predictor of the risk of MRONJ. A CTX level of less than 150 pg / mL had a sensitivity of 100% and a specificity of 81%. Bayesian analysis provides an expected risk for the MRONJ population of 0.29% (95% confidence interval, 0.12-0.52); the expected risk is 0.42% for the CTX level of less than 150 pg / mL and 0.13% for the CTX level higher than 150 pg / mL. A CTX level of less

than 150 pg / mL was sensitive and is associated with an approximately 3-fold greater risk of MRONJ [13-19].

In this study osteocalcin is normal in both groups but patients with values below 5.4 ng / ml are 3.2 times more likely to develop osteonecrosis of the jaw. The C-terminal telopeptide of mature type 1 collagen (CTX) is also normal in both groups and in MRONJ + is in the lowest quad. Values below 150 pg / ml will make the risk of osteonecrosis of the jaw about 1.92 times greater.

Conclusion

1. Low vitamin D is found in 100% of patients, low osteocalcin in 32% and low normal (lowest quartile) C-terminal telopeptide of mature type 1 collagen (CTX) in 85% of patients. Vitamin D is low in both MRONJ + and MRONJ- patients, with no significant difference between the two groups.
2. Osteocalcin is normal in both groups but patients with values below 5.4 ng / ml are 3.2 times more likely to develop osteonecrosis of the jaw. The C-terminal telopeptide of mature type 1 collagen (CTX) is also normal in both groups and in MRONJ + is in the lowest quartile. Values below 150 pg / ml make the risk of osteonecrosis of the jaw about 1.92 times greater.
3. There is a high correlation between the concentrations of vitamin D and osteocalcin (r = 0.64) and between osteocalcin and CTX (r = 0.58).

References

1. Khosla S, Burr D, Cauley J, *et al*. Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2007; 22(10):1479-1491.
2. Ruggiero SL, Thomas B. Dodson John Fantasia, Reginald Goodday, Tara Aghaloo Mehrotra.. Maxillofacial Surgeons Position Paper on Medication-Related Osteonecrosis of the Jaw—2014 Update J Oral Maxillofac Surg. 2014; 72:1938-1956.
3. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg. 2003; 61(9):1115-1117.
4. Estilo CL, Van Poznak CH, Williams T, *et al*. Osteonecrosis of the maxilla and mandible in patients with advanced cancer treated with bisphosphonate therapy. Oncologist. 2008; 13(8):911-920.
5. Marx RE, Sawatari Y, Fortin M, Broumand V. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. J Oral Maxillofac Surg. 2005; 63(11):1567-1575.
6. Otto S, Abu-Id MH, Fedele S, *et al*. Osteoporosis and bisphosphonates-related osteonecrosis of the jaw: not just a sporadic coincidence—a multi-centre study. J Craniomaxillofac Surg. 2011; 39(4):272-277.
7. Sedghizadeh PP, Stanley K, Caligiuri M, *et al*. Oral bisphosphonate use and the prevalence of osteonecrosis of the jaw: an institutional inquiry. J Am Dent Assoc. 2009; 140(1):61-66.
8. Rizzoli R, Burlet N, Cahall D, *et al*. Osteonecrosis of the jaw and bisphosphonate treatment for osteoporosis. Bone. 2008; 42(5):841-847.
9. Shibahara T, Morikawa T, Yago K, Kishimoto H, Imai Y, Kurita K. National Survey on Bisphosphonate-Related Osteonecrosis of the Jaws in Japan. Osteoporos Int. 2018; 29(10):2315-2321.
10. Koch F, Yekta SS, Merkel C, *et al*. The impact of bisphosphonates on the osteoblast proliferation and Collagen gene expression in vitro. Head Face Med, 2010, 12.
11. Niyibizi C, Eyre DR. Structural characteristics of cross-linking sites in type V collagen of bone. Chain specificities and heterotypic links to type I collagen. Eur J Biochem. 1994; 224(3):943-950.
12. Simon MJ, Niehoff P, Kimmig B, *et al*. Expression profile and synthesis of different collagen types I, II, III, and V of human gingival fibroblasts, osteoblasts, and SaOS-2 cells after bisphosphonate treatment. Clin Oral Investig. 2010; 14(1):51-58.
13. Kim JW, Kong KA, Kim SJ, Choi SK, Cha IH, Kim MR. Prospective biomarker evaluation in patients with osteonecrosis of the jaw who received bisphosphonates. Bone. 2013; 57:201-5.
14. Lorenzo-Pouso A, Pérez-Sayáns M, González-Palanca S, Chamorro-Petronacci C, *et al*. Biomarkers to predict the onset of biphosphonate-related osteonecrosis of the jaw: A systematic review Med Oral Patol Oral Cir Bucal. 2019; 24(1):e26-e36.
15. Marx RE1, Cillo JE, Ulloa JJ. Oral bisphosphonate-induced osteonecrosis: risk factors, prediction of risk using serum CTX testing, prevention, and treatment. J Oral Maxillofac Surg. 2007; 65(12):2397-410.
16. Kanety H, Givol N, Yahalom R, Taicher S, Yarom N.J Assesing serum C-telopeptide cross-link of type 1 collagen in BONJ.Oral Maxillofac Surg. 2014; 72(8):1456-6266.
17. Hutcheson A, Cheng A, Kunchar R, Stein B, Sambrook P, Goss A. A C-terminal crosslinking telopeptide test-based protocol for patients on oral bisphosphonates requiring extraction: a prospective single-center controlled study. J Oral Maxillofac Surg, 2010.
18. Enciso R, Keaton J, Saleh N, Ahmadieh A, Clark GT, Sedghizadeh PP. Assessing the utility of serum C-telopeptide cross-link of type 1 collagen as a predictor of bisphosphonate-related osteonecrosis of the jaw: A systematic review and meta-analysis.
19. Dal Prá KJ1, Lemos CA2, Okamoto R3, Soubhia AM4, Pellizzer EP2.Efficacy of the C-terminal telopeptide test in predicting the development of bisphosphonate-related osteonecrosis of the jaw: a systematic review.J Am Dent Assoc. 2016; 147(7):551-560.
20. Santini D, Vincenzi B, Avvisati G, *et al*. Pamidronate induces modifications of circulating angiogenetic factors in cancer patients. Clin Cancer Res. 2002; 8(5):1080-1084.
21. Walter C, Klein MO, Pabst A, *et al*. Influence of bisphosphonates on endothelial cells, fibroblasts, and osteogenic cells. Clin Oral Investig. 2010; 14(1):35-41.
22. Landesberg R, Cozin M, Cremers S, *et al*. lInhibition of Oral Mucosal Cell Wound Healing by Bisphosphonates J Oral Maxillofac Surg. 2008; 66:839-847.
23. Hoefert S, Schmitz I, Tannapfel A, Eufinger H. Importance of microcracks in etiologyof bisphosphonate-related osteonecrosis of the jaw:a possible pathogenetic model of symptomaticand non-symptomatic osteonecrosis of the jaw based on scanning electron microscopy findings. Clin Oral Invest. 2010; 14:271-284.

24. Mashiba T, Mori S, Burr DB, *et al*. The effects of suppressed bone remodeling by bisphosphonates on microdamage accumulation and degree of mineralization in the cortical bone of dog rib. J Bone Miner Metab. 2005; 23(suppl):36-42.
25. Heim N, Warwas F, Wilms C, Reich R, Markus V. Vitamin D (25-OHD) deficiency may increase the prevalence of medication-related osteonecrosis of the jaw J Oral Maxillofac Surg. 2017; 46(2):151-156.
26. Bedogni A, Bettini G, Bedogni G, Basso D, Gatti D, Valisena S, et al. Is vitamin D deficiency a risk factor for osteonecrosis of the jaw in patients with cancer? A matched case-control study. J Craniomaxillofac Surg. 2019 S1010-5182(18)31039-4.
27. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ. 1996; 312(7041):1254-1259.
28. Schuit SC, van der Klift M, Weel AE, *et al*. Fracture incidence and association with bone mineral density in elderly men and women: the Rotterdam Study. Bone. 2004; 38(4):195-202.
29. Kanis JA, Johnell O, De Laet C, *et al*. A meta-analysis of previous fracture and subsequent fracture risk. Bone. 2004; 35(2):375-382.
30. Plaza SM, Lamson DW. Vitamin K2 in bone metabolism and osteoporosis. Altern Med Rev. 2005; 10(1):24-35.
31. Shearer MJ, Newman P. Metabolism and cell biology of vitamin K. Thromb Haemost. 2008;100(4):530-547.
32. Davidson RT, Foley AL, Engelke JA, Suttie JW. Conversion of dietary phylloquinone to tissue menaquinone-4 in rats is not dependent on gut bacteria. J Nutr. 1998; 128(2):220-223.
33. Ronden JE, Drittij-Reijnders MJ, Vermeer C, Thijssen HH. Intestinal flora is not an intermediate in the phylloquinone-menaquinone-4 conversion in the rat. Biochim Biophys Acta. 1998; 1379(1):69-75.
34. Thijssen HH, Drittij-Reijnders MJ, Vitamin K. distribution in rat tissues: dietary phylloquinone is a source of tissue menaquinone-4. Br J Nutr. 1994; 72(3):415-425.
35. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest. 1993; 91(4):1769-1774.
36. Binkley NC, Krueger DC, Engelke JA, *et al*. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. Am J Clin Nutr. 2000; 72(6):1523-1528.
37. Yasui T, Miyatani Y, Tomita J, *et al*. Effect of vitamin K2 treatment on carboxylation of osteocalcin in early postmenopausal women. Gynecol Endocrinol. 2006; 22(8):455-459.
38. Van Summeren MJ, Braam LA, Lilien MR, *et al*. The effect of menaquinone-7 (vitamin K2) supplementation on osteocalcin carboxylation in healthy prepubertal children. Br J Nutr. 2009; 102(8):1171-1178.