Alendronate Sodium in Osteoarthritis: Effects on Lipid Profile, Circulating Leptin and the Clinical Activity

Authors

Sinaa Abdul Amir Kadhim¹, Haidar Mahdi Jawad², Sami Salman Shihab³

¹Ass. Prof., Pharmacology Department, College of Medicine, University of Al-Qadisiyah Iraq

Email: sinaa.kadhim@qu.edu.iq

²Ass. Prof in Pharmacology Department, College of Medicine, University of Baghdad Iraq

Email: haidaralshakarchi@yahoo.com

³Professor, College of Medicine, University of Baghdad, Iraq

Email: ssshihab2@gmail.com

ABSTRACT

Objective: Osteoarthritis (OA) is a most common arthritic disease and responsible for more of disability in all world. OA affects all joint and characterized by degredation of cartilage, subchondral bone turnover and osteophytes formation.

Aim of the study: To evaluate the effectiveness of alendronate sodium (ALN) on disease activity and physical function, evaluate the lipid profile and study the impact of ALN on circulating leptin marker and myeloperoxidase (MPO) and to determine its effectiveness in slowing progression of disease.

Patients and Methods: 116 OA patients over 45 years old with X-ray grade II and more of Kellgren and Lawrence grading were enrolled in our study. Base line assessment was done, Kellgren and Lawrence X-ray grading, WOMAC scoring, body mass index and the lipid profil with enzyme-linked immunosorbent assay (ELISA) analysis of serum leptin and MPO. They were instructed to take ALN 10 mg daily. Reassessment was done after 3 months.

Results: A significant symptomatic improvement in WOMAC scoring (pain, stiffness) were observed associated with significant reduction in serum leptin and no significant reduction in MPO. A nonsignificant changes in lipid profile, joint space width and function were also reported.

Conclusion: ALN in patients with OA has clinical efficacy in reducing suffering especially pain probably through inhibition of leptin with no significant joint structural improvement, and may help delay and prevent further disease progression probably through inhibition of leptin activity.

Introduction

Osteoarthritis (OA) is a progressive degeneration of articular cartilage with subsequently joint space width (JSW) narrowing (Blagojevic *et al.*, 2010), a most common form of joint disorders (Bijlsma

et al., 2011). OA occurrence includes reduction in mineralization of the trabecular structure, associated with destruction in the subchondral bone, defects in bone marrow and subchondral plate sclerosis (Karsdalet *et al.*, 2014). This resulted

in a reduction in JSW leading to immobile, and disabling joint (Kon et al., 2012). OA can be classified as primary (idiopathic) if its cause is not well defined and secondary when there is a certain events causing OA.(Brandt *et al.*, 1986). The initiation of OA may occur under influence of many factors like genetic, local, systemic and environmental factors (Brandt et al., 2009). OA affected group of joint as joint of end of finger, base of thumb, neck, knee, base of big toe, lower back and hip (Hochberg, 2001). Healthy lifestyle measures in women are essential to permit healthy ageing, and the antioxidants lead to pain relief and functional improvement in knee OA, in many things, were based on avocado-soya bean and curcumin (Grover and Samson, 2016).

OA is a degenerative joint abnormality (Blagojevic *et al.*, 2010) which may associated with inflammatory events (Berenbaum, 2013). Pathophysiological changes in the synovial fluid may also occur, as well as subchondral bone and other joint structures (Suri *et al.*, 2012).

Recently OA associated with increased activity of different cytokines in the joints (Loeser *et al.*, 2012) which lead to production of enzymes that mediate destruction of cartilage (Goldring and Goldring, 2007). The synovium is a main part for gross and microscopic inflammatory changes (Sellam and Berenbaum, 2010). Increasingly recognized is the presence of synovitis in a significant value in primary OA (Jeremy and Christin, 2013). Synovitis is occur following cartilage destruction and shows a critical link in the processes of initiation and progression of OA (Scanzello and Goldring, 2012).

Leptin is a nonglycosylated protein encoded by ob (obese) gene express in OA, is secreted by adipose tissue, so it is higher in over weight persons (de Boer et al., 2012). It represent a causative a link between obesity and osteoarthritis (Scotece and Mobasher, 2015) and act as a body weight homeostasis, immune and inflammatory processes, including increase MMP production, regulator (Otero *et al.*, 2006). Leptin with its receptor are

present in chondrocytes, synovium, osteophytes and infrapatellar fat pad (Presle *et al.*, 2006). It has a biphasic activity, it contributing to cartilage synthesis when leptin is low and facilitates cartilage degradation at high level (Pottier *et al.*, 2006). Leptin induces chondrocytes apoptosis (Huang *et al.*, 2016). It has been discovered that leptin mediated the associations between adiposity measures and cartilage thickness, and it may have a key role in cartilage thinning (Oliver *et al.*, 2015). Such findings have concluded the importance of leptin as a therapeutic target for the treatment of OA in the overweight population (Zhang *et al.*, 2016 ; Huang *et al.*, 2016).

Myeloperoxidase (MPO) is a peroxidase enzyme , express in humans neutrophil, oxidatively damaging host tissue (Klebanoff, 2005). MPO may be present in the synovial fluid of OA (Steinbeck *et al.*, 2007). In OA serum, MPO level reduced after joint replacement (Deberg et al., 2008). Thus MPO may serve as diagnostic marker for detection of early OA (Steinbeck et al., 2007).

OA sign and symptoms, including pain, tenderness, decrease flexibility, stiffness, harsh sensation and bone overgrowth, often increase slowly and get worse over time and vary among OA patients (Srikulmontree, 2012 ; Zhang *et al.*, 2010). Use-related pain in OA is frequent but rest pain and pain at night also occur, and a many patterns of pain can be seen, varying from a dull ache to sharp, stabbing pain (Hawker *et al.*, 2008), The Western Ontario and McMaster Universities Arthritis Index (WOMAC) scores were more in the diffuse compared to anterior-medial patterns OA pain (Gincke *et al.*, 2016).

Routine laboratory investigations, in patient with OA, appear to be within normal ranges. Primary OA is typically diagnosed according to clinical and radiographic imaging findings (Hunter, 2008). Kellgren and Lawrence (KL) system is the most often used (Kellgren and Lawrence, 1957) in form of following grading

Grade	Score	Description
None	0	clear-cut absence of x-ray changes
Doubtful	1	possible osteophytic lipping
Minimal	2	clear-cut osteophytes and possible tightening of joint space
Moderate	3	moderate numerous osteophytes, clear-cut tightening of joints space, a few sclerosis and possible abnormality of bone contour
Severe	4	great osteophytes, obvious narrowing of joint space, rigorous sclerosis and clear-cut deformity of bone contour

It has been discovered that a combination of both pharmacological and non-pharmacological therapies exert a more beneficial role in treating patients with knee osteoarthritis (Alshami, 2014). many drugs is used in treatment of OA like acetaminophen, NSAID, calcitonin, glucosamine and more with not so clear advantages as disease modifying activity. Alendronate sodium the antiresorptive drug appears in the field now a day. It is an anohormonal therapeutic agent, synthetic analogs of pyrophosphate attach to the hydroxyapatite, one of bone contents. It considered as a member of bisphosphonates. ALN interfere with normal bone formation and turnover cycle in the body by slowing bone loss with elevating bone mass by interfering with osteoclast (Kosuke *et al.*, 2010). The bisphosphonate inhibitory action on bone resorption is resulted from accumulation of bisphosphonate in osteoclasts after released from bone surfaces during bone resorption. Bisphosphonates inhibit farnesyl pyrophosphate synthase (Michael *et al.*, 2011). It has been demonstrated that ALN has the ability to protect chondrocyte from OA events induced by IL-1 β (Wang *et al.*, 2011) by increasing Collagen II and reduction of MMP-13 within chondrocytes (Wang *et al.*, 2011). ALN is used in osteoporotic postmenopausal lady (Nijset *et al.*, 2006), male osteoporosis (Orwollet *et al.*, 2010), corticosteroid-associated osteoporosis (Nijs *et al.*, 2006), paget's disease (Reid and Hosking., 2011) and treatment for osteogenesis imperfecta (Atsuko *et al.*, 2016). More recent clinical study, early starting of treatment with anti-resorptive agent gives better outcome which play a valuable

role in pain reduction and disease modification of osteoarthritis (Laslett *et al.*, 2014).

A potential benefit of antiresorptive agents, bisphosphonates, results from experimental studies have shown promising results in treatment of OA (Spector, 2003). ALN intake, in symptomatic hip OA, is effective in pain reduction (Nishii *et al.*, 2013). ALN, in randomized controlled trial, was associated with less spinal osteophyte and joint space narrowing progression (Neogi *et al.*, 2008). ALN, in animal model of severe OA study, reduced subchondral bone turnover, reduced osteophyte formation and suppression of articular cartilage degeneration, so ALN reduces OA progression, with suggestion ALN has chondroprotective effects in OA (Siebelt *et al.*, 2014; Mohan *et al.*, 2013). Both in vitro and in vivo studies, it has been found that ALN has the ability to protect chondrocytes by decreasing MMP-13 expression (Hu *et al.*, 2009).

Preclinical trail of rabbit model of OA, has found that ALN prevented periarticular loss of bone (Shirai *et al.*, 2011), decrease the expression of matrix metalloproteinase-13 and interleukin-1 β and it has been suggested that ALN had a chondroprotective effect in OA (Shirai *et al.*, 2011).

Patients and Method

116 OA patients over 45 years old with Kellgren and Lawrence X-ray grade 2 and more, the male patients were 32 while the female were 84 giving the male to female ratio of 1:2.63 and mean BMI (kg/m²) was 30.21 $\pm$ 5.91, were enrolled in this study. The patients were habitants of the city of Al-Diwanyhia and Baghdad and had the Iraq nationality. Laboratory equipment and reagents were of the highest available grades. Base line assessment were done in form Kellgren and Lawrence X-ray grading (Kellgren and Lawrence, 1957), WOMAC scoring (Falk *et al.*, 2008), body mass index and the biochemical parameters. Reassessment was done after 3 months of treatment. Measurement of serum lipid profile, MPO (ELISA), leptin (ELISA), WOMAC and KL

were done. Numeric variables were expressed as mean standard deviation. Comparison was done using paired t-test.

Results : Table (1) Demographic characteristic of the study population

Characteristic	N	Mean $\pm$ SD	Range
Sample size	116		
Age (years)		54.68 $\pm$ 5.16	45-65
Gender (m/f)	32/84 (1:2.63)		
BMI (kg/m ²)		30.21 $\pm$ 5.91	21.87-37.81

n: number of cases; m/f: male to female ratio; BMI: Body Mass Index

The results were as follows: mean serum cholesterol showed no significant change, 188.97 $\pm$ 44.36 versus 186.27 $\pm$ 42.78 (P=0.131); mean serum TG showed no significant change (elevation), 132.45 $\pm$ 64.19 versus 131.72 $\pm$ 56.66 (P=0.829); no significant alteration in mean serum HDL, 40.27 $\pm$ 6.63 versus 40.12 $\pm$ 6.86 (P=0.693); no significant change(reduction) in mean serum LDL, 122.39 $\pm$ 40.06 versus 123.42 $\pm$ 34.19 (P=0.768), as shown in table (2) and figures (1) through (4).

Figure (1): Mean cholesterol level before and after treatment with ALN

Figure (2): Mean TG level before and after treatment with ALN

Figure (3): Mean HDL level before and after treatment with 10 mg ALN

Figure (4): Mean LDL level before and after treatment with 10 mg ALN

Mean JSW score was not significantly changed after treatment, 2.76 $\pm$ 0.59 versus 2.80 $\pm$ 0.53 (P=0.295). The mean score of pain showed highly significant reduction from 7.93 $\pm$ 1.38 to 5.41 $\pm$

1.29 ($P < 0.001$); the same was observed for function score which was reduced, insignificant, from 40.39 ± 6.10 to 39.54 ± 6.51 ($P = 0.661$). On the other hand Stiffness score was shown to be reduced (highly significant reduction) from 3.90 ± 1.20 to 2.22 ± 1.14 ($P < 0.001$)

Figure (5): Mean JSW before and after treatment with ALN

Figure (6): Mean pain score level before and after treatment with ALN

Figure (7): Mean function score level before and after ALN

Figure (8): Mean stiffness score level before and after with ALN.

(*) means significant

Mean serum leptin showed highly significant reduction, 1369.50 ± 564.90 versus 1067.60 ± 454.89 ($P < 0.001$). On the other hand mean MPO was no significantly changed before and after treatment, 0.81 ± 0.23 versus 0.74 ± 0.45 ($P = 0.100$).

Figure (9): Mean Leptin level before and after treatment with ALN

Figure (10): Mean MPO level before and after treatment with ALN

Discussion

The importance of pharmacological therapy of OA is reducing pain and cartilage protection. we demonstrated that there was clinical improvement with no observed radiological improvement. Significant alteration in Western Ontario and McMaster Universities (WOMAC) scores together with no significant changes in Kellgren-Lawrence grade (KL) joint space width (JSW) were obtained by the current study. WOMAC gives an acceptable measure of the degree of knee pain affected by (Jinks *et al.*, 2002). The present study showed that ALN use was associated with reduced knee pain severity as assessed by WOMAC scores. In agreement with our finding, Carbone and coworkers in 2004 reported significant change in pain according to WOMAC score in patients with OA after 3 years treatment with ALN. In agreement with current results, placebo control trial has found that there was a substantial improvement in total WOMAC score (Jokar *et al.*, 2010). Nishii and coworkers in 2013 suggested that 2 years ALN intake is effective in pain reduction associated with no significant observation obtained in OA changes as precluded by radiological JSW. All of the trials have stated that there was significant reduction in urinary CTX-II levels in all enrolled groups. Pain suppression in OA may be also due to suppression of Matrix metalloproteinase (MMP) -13, a cytokine not involved in our study, by ALN (Hu *et al.*, 2009).

The alteration in serum lipid profile (Tc, TG, HDL and LDL) was not significant in the current study after treatment with ALN. The lack of significant alteration in serum lipid profile is an indicator that dyslipidemia is not a problem with the use of ALN and that there is no significant increase in cardiovascular and cerebrovascular risk in patients using it for OA. In agreement with the results reported by several other observational studies on human and several experimental studies on experimental animals (Sanad *et al.*, 2011; Iwamoto *et al.*, 2008; Sookvanichsilpet *et al.*, 2014). According to the result of the present study, the

serum leptin was significantly reduced following treatment with ALN. Following the discovery of leptin receptors in osseous tissue several studies have been carried out to investigate its involvement in osseous metabolism. Some researchers have shown that leptin is expressed and secreted from human osteoblasts primary cultures during the period of mineralization, and that it may activate osteogenesis in human bone marrow in vitro (Reseland *et al.*, 2001). Recent study in OA has recorded that progression of Kellgren-Lawrence grade and high levels of plasma leptin were found to increase knee OA severity (Calvet *et al.*, 2016; Staikos *et al.*, 2013). Other study has demonstrated that severe pain was significantly correlated with high synovial fluid content of leptin in hip and knee OA (Bas *et al.*, 2014).

The synovial fluid and plasma leptin might be a markers correlated with the severity of knee OA (Staikos *et al.*, 2013). Authors has found that in addition to leptin levels in the joint; leptin sensitivity in the cartilage are also increased in over weight patients with OA (Vuolteenaho *et al.*, 2014). This findings referring to leptin as a causative bridge between OA and obesity and that lowering of leptin is a target to the formation of disease-modifying therapeutics for OA (Vuolteenaho *et al.*, 2014). It has been found that joint pain correlated with increased synovial fluid leptin concentrations and raised pre-operative pain seen in obese may be linked to high intra-articular leptin concentrations (Lübbecke *et al.*, 2013). In contrast to current study, other authors observed, after 12 months ALN treatment, that serum leptin was not affected by treatment; however this study included a sample of postmenopausal women treated for osteoporosis (Sebastián-Ochoa *et al.*, 2012). Pre-clinical studies have demonstrated that leptin administration to Ovariectomized rats resulted in reduced bone turnover, playing role in OA, and they concluded that leptin can inhibit or at least delay osteoporosis (Abdel-Sater and Mansour, 2013). Based on these observations, targeting leptin may play a role in treating OA

patients.

The present study showed that myeloperoxidase MPO was not affected by ALN treatment. It was shown in other clinical study that serum MPO is higher in patients with erosive OA than patients with non-erosive subtype (Punzi, 2012) and patients with erosive type may not benefit completely from ALN treatment and targeting MPO may be considered using other modality of treatment for that subset of patients.

Conclusion

The use of Alendronate in patients with osteoarthritis has clinical efficacy in reducing symptoms especially pain probably through inhibition of leptin, and MMP-13 with no structural improvement and may help delay and prevent further disease progression probably through inhibition of leptin activity. Alendronate proved to be safe in old patients with dyslipidemia since there is no associated lipid disturbances.

References

1. Abdel-Sater K and Mansour H. Bone biomarkers of ovariectomised rats after leptin therapy. Bratisl Lek Listy. 2013; 114(6):303-307.
2. Abramson S, Attur M, Yazici Y. Prospects for disease modification in osteoarthritis. Nat Clin Pract Rheumatol 2006;2:304–12.
3. Alshami A. Knee osteoarthritis related pain: a narrative review of diagnosis and treatment. Int J Health Sci (Qassim). 2014 Jan; 8(1): 85–104.
4. Atsuko S, Jean O, Takeshi M, et al. Scoliosis in osteogenesis imperfecta caused by *COL1A1/ COL1 A2* mutations — genotype–phenotype. correlations and effect of bisphosphonate treatment Bone May 2016 Volume 86, Pages 53–57.
5. Bijlsma J, Berenbaum F, Lafeber F. Osteoarthritis: an update with relevance for clinical practice. Lancet 2011; 377:2115–26.
6. Blagojevic M, Jinks C, Jeffery A et al. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. Osteoarthritis Cartilage. 2010, 18: 24-33.
7. Brandt K, Dieppe P, Radin E. "Etiopathogenesis of osteoarthritis". Med. Clin. North Am. January 2009 93 (1): 1–24.
8. Brandt K, Mankin H, Shulman L. Workshop on etiopathogenesis of osteoarthritis, J Rheumatol 1986,13, 1126-1160.
9. Calvo E, Castaneda S, Largo R, et al. Osteoporosis increases the severity of cartilage damage in an experimental model of osteoarthritis in rabbits. Osteoarthritis Cartilage 2007;15:69–77.
10. Carbone L, Nevitt M, Wildy K, et al. The relationship of antiresorptive drug use to structural findings and symptoms of knee osteoarthritis. Arthritis Rheum 2004; 50(11): 3516-3525.
11. De Boer T, Van Spil W, Huisman A, et al. Serum adipokines in osteoarthritis ;comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. Osteoarthritis Cartilage 2012.
12. Deberg M, Dubuc J, Labasse A, et al. One-year follow-up of Coll2-1, Coll2-1NO₂ and myeloperoxidase serum levels in osteoarthritis patients after hip or knee replacement. Ann Rheum Dis 2008;67:168-174.
13. Falk A, Benbouazza K, Amine B, et al. Translation and validation of Moroccan Western Ontario and McMaster Universities (WOMAC) osteoarthritis index in knee osteoarthritis. Rheumatol Int. 2008;28:677-683.
14. Ginckel A, Bennell K, Campbell P, et al. Location of knee pain in medial knee osteoarthritis: patterns and associations with self-reported clinical symptoms. Osteoarthritis and Cartilage July 2016 Volume 24, Issue 7, Pages 1135–1142.

13. Goldring M, Goldring S. Osteoarthritis. *J Cell Physiol.* 2007; 213(3) : 626–634.
14. Grover A, Samson S. Benefits of antioxidant supplements for knee osteoarthritis: rationale and reality. *Nutr J.* 2016 Jan 5;15:1.
15. Hawker G, Stewart L, French M, et al. Understanding the pain experience in hip and knee osteoarthritis—an OARSI/OMERACT initiative. *Osteoarthritis Cartilage.* 2008;16(4):415–22
16. Hochberg M. Osteoarthritis, B. Clinical features. In :Klippel JH, Crofford LJ, Stone JH, Weyand CM, eds. *Primer on the Rheumatic Diseases*, 12th ed. Atlanta, Georgia :Arthritis Foundation, 2001, 289–293.
17. Huang Z, Du S, Huang L, et al. Leptin promotes apoptosis and inhibits autophagy of chondrocytes through upregulating lysyl oxidase-like 3 during osteoarthritis pathogenesis. *Osteoarthritis Cartilage.* 2016 Jul; 24(7):1246–1253.
18. Huang Z, Li J, Du S et al. One. Effects of UCP4 on the Proliferation and Apoptosis of Chondrocytes: Its Possible Involvement and Regulation in Osteoarthritis. 2016; 11(3):e0150684.
19. Hunter D. Advanced imaging in osteoarthritis. *Bull NYU HospJt Dis.* 2008. 66(3):251–60.
20. Hu H, Zhang L, Li B, et al. In vitro effect of alendronate on chondrocytes and articular cartilage and subchondral bone in rabbit anterior cruciate ligament transection model. *Zhongguo Xiu Fu Chong Jian WaiKe ZaZhi.* 2009 Dec; 23(12):1474–81.
21. Iwamoto J., Sato Y, Uzawa M, Takeda T, et al. Comparison of effects of alendronate and raloxifene on lumbar bone mineral density, bone turnover, and lipid metabolism in elderly women with osteoporosis,” *Yonsei Medical Journal* 2008; 49 (1): 119–128.
22. Jinks C, Jordan K, Croft P. Measuring the population impact of knee pain and disability with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). *Pain* 2002; 100: 55–64.
23. Jokar M, Mirfeizi Z, Keyvanpajoh K. The Effect of Alendronate on Symptoms of Knee Osteoarthritis: A Randomized Controlled Trial. *Iranian Journal of Medical Sciences IJMS* (2010) Vol 35, No 1:9–15.
24. Karsdal M , Bay-Jensen A , Lories R, et al. The coupling of bone and cartilage turnover in osteoarthritis: opportunities for bone antiresorptives and anabolics as potential treatments? : *Ann Rheum Dis* 2014;73:336–348.
25. Kellgren J, Lawrence J. Radiological assessment of osteoarthrosis. *Ann Rheum Dis.* 1957;16:494–502.
26. Kon E, Filardo G, Drobnic M, et al. Madry H, Jelic M, Dijk N, et al. Non-surgical management of early knee osteoarthritis. *Knee Surg Sports Traumatol Arthrosc.* 2012;20:436–9.
27. Kosuke K, Hidemasa K, Mari A, et al. Development of a novel transdermal patch of alendronate, a nitrogen-containing bisphosphonate, for the treatment of osteoporosis. *Journal of Bone and Mineral Research* Volume 25, Issue 12. December 2010 Pages 2582–2591.
28. Lafyatis R, York M, Marshak-Rothstein A. Antimalarial agents. closing the gate on Toll-like receptors? *Arthritis Rheum.* (2006);54:3068–3070.
29. Lampropoulou K, Dontas I, Stathopoulos I, et al. Chondroprotective effect of high-dose zoledronic acid: an experimental study in a rabbit model of osteoarthritis. *J Orthop Res*, 32 (2014), pp. 1646–1651.
30. Laslett L, Kingsbury S, Hensor E, et al. Effect of bisphosphonate use in patients with symptomatic and radiographic knee osteoarthritis: data from the Osteoarthritis

- Initiative. *Ann Rheum Dis*, 73 (2014), pp. 824–830.
31. Loeser R, Goldring S, Scanzello C, et al. Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum*. 2012;64(6):1697–1707.
 32. Lübbecke A, Finckh A, Puskas G, et al. Do synovial leptin levels correlate with pain in end stage arthritis?. *IntOrthop*. 2013 Oct; 37(10):2071-9. Epub 2013 Jul 9.
 33. Mahjoub M, Berenbaum F, Houard X. Why subchondral bone in osteoarthritis? The importance of the cartilage bone interface in osteoarthritis. *Osteoporos Int*. 2012 ;23(Suppl 8):841–846.
 34. Michael J, Julie C, Fraser P, et al. Biochemical and molecular mechanisms of action of bisphosphonates. *Bone* Volume 49, Issue 1, July 2011, Pages 34–41.
 35. Mohan G, Perilli E, Parkinson I. et al. Pre-emptive, early, and delayed alendronate treatment in a rat model of knee osteoarthritis: effect on subchondral trabecular bone microarchitecture and cartilage degradation of the tibia, bone/cartilage turnover, and joint discomfort. *Osteoarthritis Cartilage*. 2013 Oct;21(10):1595-604.
 36. Neogi M, Ensrud K, Bauer D, et al. The effect of alendronate on progression of spinal osteophytes and disc-space narrowing. *Ann Rheum Dis* 2008;67:1427-1430.
 37. Nijs R, Jacobs J, Lems W, et al. Alendronate or Alfacalcidol in Glucocorticoid-Induced Osteoporosis *N Engl J Med* 2006; 355:675-684 August 17, 2006.
 38. Nishii T, Tamura S, Shiomi T, et al. Alendronate treatment for hip osteoarthritis: prospective randomized 2-year trial. *Clin Rheumatol* (2013); 32(12): pp. 1759–1766.
 39. Oliver P, Yuelong C, Benny A, et al. Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. *Ann Rheum Dis* 2015;74:82–88.
 40. Orwoll E, Miller P, Adachi J, et al. Efficacy and safety of a once-yearly i.v. Infusion of zoledronic acid 5 mg versus a once-weekly 70-mg oral alendronate in the treatment of male osteoporosis: A randomized, multicenter, double-blind, active-controlled study. *JBM* Volume 25, Issue 10 October 2010 Pages 2239–2250. 9:21–6.
 41. Pottie P, Presle N, Terlain B, et al. Obesity and osteoarthritis: more complex than predicted!. *Ann Rheum Dis* 2006;65: 1403–1405.
 42. Presle N, Pottie P, Dumond H, et al. Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. *Osteoarthritis Cartilage* 2006;14:690–5.
 43. Punzi L. Coll2-1, Coll2-1NO2 and myeloperoxidase serum levels in erosive and non-erosive osteoarthritis of the hands. *Osteoarthritis Cartilage* 2012; 20:557e61.
 44. Reid I and Hosking D. Bisphosphonates in Paget's disease. *Bone* Volume 49, Issue 1, July 2011, Pages 89–94.
 45. Reseland J, Syversen U, Bakke I, et al. Leptin is expressed in and secreted from primary cultures of human osteoblasts and promotes bone mineralization. *Journal of Bone & Mineral Research* 2001; 16: 1426–1433.
 46. Scanzello C and Goldring S. The role of synovitis in osteoarthritis pathogenesis. *Bone*. 2012 Aug; 51(2):249-57. Scotece M and Mobasher A. Leptin in osteoarthritis: Focus on articular cartilage and chondrocytes. *Life Sci*. 2015 Nov 1;140:75-8.
 47. Sebastián-Ochoa A, Fernández-García D, Reyes-García R, et al. Adiponectin and leptin serum levels in osteoporotic postmenopausal women treated with

- raloxifene or alendronate. Menopause. 2012;19:172–177.
48. Sellam J and Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat Rev Rheumatol. 2010;6(11):625–635.
 - Shirai T, Kobayashi M, Nishitani K, et al. Chondroprotective effect of alendronate in a rabbit model of osteoarthritis. J Orthop Res. 2011 Oct;29(10):1572-7.
 49. Siebelt M, Waarsing J, Groen H, et al. Inhibited osteoclastic bone resorption through alendronate treatment in rats reduces severe osteoarthritis progression. Bone, 66 (2014), pp. 163–170.
 50. Sookvanichsilp N, Tad-urai N, Lawtong P, et al. Effects of Antiresorptive Drugs on Long Bone Growth, Body Weight Gain, and Serum Lipid Levels in Young Adult Ovariectomized Rats. Mahidol University Journal of Pharmaceutical Sciences 2014; 41 (4), 29-38.
 51. Spector T. Bisphosphonates: Potential therapeutic agents for disease modification in osteoarthritis Aging Clinical and Experimental Research October 2003, Volume 15, Issue 5, pp 413-418.
 52. Steinbeck J, Nesti J, Sharkey P, et al. Myeloperoxidase and chlorinated peptides in osteoarthritis: potential biomarkers of the disease. Journal of Orthopedic Research Volume 25, Issue 9 September 2007 Pages 1128–1135.
 53. Suri P, Morgenroth D, Hunter D. Epidemiology of osteoarthritis and associated comorbidities. PM R J. 2012, 4: S10 S19.10.
 54. Vuolteenaho K, Koskinen A, Moilanen E. Leptin - a link between obesity and osteoarthritis. applications for prevention and treatment. Basic Clin Pharmacol Toxicol. 2014 Jan;114(1):103-8. doi: 10.1111/bcpt.12160. Epub 2013 Nov 20.
 55. Wang Z, Wang W, Zhang L, et al . Effect of alendronate on interleukin-1beta induced chondrocytes of rat in vitro. ZhongguoXiu Fu Chong JianWaiKeZaZhi. 2011Jan;25(1):50-5.
 56. Zhang W, Doherty M, Peat G, et al. EULAR evidence-based recommendations for the diagnosis of knee osteoarthritis. Ann Rheum Dis. 2010;69:483–9.
 57. Zhang ZM, Shen C, Li H, et al. Leptin induces the apoptosis of chondrocytes in an in vitro model of osteoarthritis via the JAK2-STAT3 signaling pathway. Mol Med Rep. 2016 Apr; 13(4):3684-90.