ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Assessment of the Correlation between M-CSF and GM-CSF with Estrogen and BMD in Osteoporosis Postmenopausal Women.

Ahmed Moussa Almohanna[2], Sami Waheed Radhi[1], Manal F Al-Khakani[1]*.

[1] Department of Chemistry, College of Science, Kufa University, Iraq
[2] Department of Internal Medicine, College of Medicine, Kufa University, Iraq

ABSTRACT

Osteoporosis is group of those diseases which affect elderly people especially women. They have adverse effects on person and economic burden on society, because of their side effects especially hip fractures. Each year many women with those disease mainly those with osteoporosis are exposed to incidents of fracture. These incidents are merely resulting from falling or simple reasons such as the acts of bending or coughing as in the case with the vertebral fracture. Therefore, it is crucial to identify those people who are at risk, from the very early stages of such diseases. Macrophage colony stimulating factor (M-CSF) is a member of the family of proteins referred to as colony stimulating factors(CSFs).M-CSF is a secreted or a cell surface glycoprotein comprised of two subunits that are joined by a disulfide bond with a total molecular mass varying from 40 to 90 kD. Similar to other CSFs, M-CSF is produced by macrophages, monocytes, and human joint tissue cells,. M-CSF-related disease states include osteoporosis, in which monocytes/macrophages and related cell types play a role. For instance, osteoclasts are similar to macrophages and are regulated in part by M-CSF. Postmenopausal bone loss results from defective bone remodeling secondary to an uncoupling of bone formation from exuberant osteoclast mediated bone resorption as a consequence of estrogen deficiency. Multinuclear osteoclast formation induced by (M-CSF + RANKL) is completely abrogated by treatment with GM-CSF. In the present study, the correlation of M-csf and GM-CSF level with Bone mineral densityand Estrogen was investigated to determine the factors that mainly affect M-CSF correlation in patients with osteoporosis. M-CSF and other measured biochemical parameters were compared between the patients (122) and healthy (32). Serum (M-CSF) and (GM-CSF) were positively correlated ($p < 0.0001$) with Estrogen, and negatively($p < 0.001$) correlated with BMD. GM-CSF was correlated with M-CSF. showed a significant difference between osteoporotic and control groups. Estrogen and bone mineral density (BMD) levels can be predicted significantly using the most correlated with M-csf and GM-csf

Keywords: 0steoporosis, M-CSF,GM-CSF ,BMD, Estrogen.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Osteoporosis is a bone disease in which the quantity of bone is reduced and the structural integrity of trabecular bone is disturbed. Cortical bone becomes more porous and thinner. This renders the bone very weak and more likely to fracture. Osteoporosis is characterized by reduce(BMD) and enhanced likelihood of bone fracture. (1)Fracture sequela include pain psychiatric distress, stature changes, raise morbidity and mortality, and increased hospitalization.(2,3)The diagnosis of osteoporosis can be made using conventional radiography and by measuringBMD.(4*)*The most popular method of measuring BMD is DEXA. In addition to the detection of abnormal(BMD), the diagnosis of osteoporosis requires investigations into potentially modifiable underlying causes, this may be done with blood tests. Depending on the likelihood of an underlying problem, investigations for cancer with metastasis to the bone, multiple myeloma, Cushing's disease and other above-mentioned causes may be performed. measurement of BMD , radiography and biochemical markers, are important in diagnosing osteoporosis.(5)Bone density testing is used to diagnose patient suffer from osteoporosis, and x-ray films are helped to rule out other bone or arthritic conditions. Thin bones may be diagnosed on an X-ray film, but bone density testing is more accurate .It is possible to detect osteoporosis noninvasively and early. Osteoporosis may be diagnosed after fractures that happen with minimal trauma, by measurement of BMD with bone densitometry which is also known as DEXA scan, or by an incidental finding on an x-ray film(6,7).DEXA scan are considered as an instant snapshot of bone status. This scan, also known collectively as BMD test, are useful to detect the amounts of bone mass in the wrist, hip, spine, heel , hand, or the entire body and to evaluate its density. Some studies have showed that information regarding bone mineral density at any anatomic site is equally valuable for estimating the risk of fracture in general.(6,7,8)DXEA is considered the gold standard for the diagnosis of osteoporosis. Osteoporosis is diagnosed when BMD is less than or equal to 2.5 standard deviations below that of a young (30–40)year-old*,(9)*healthy adult women reference population. This is translated as a T-score. But because bone density decreases with age, more people become osteoporotic with increasing age,*(9,10)*the World Health Organization has established the following diagnostic guidelines.*(9*,11)The International Society for Clinical Densitometry takes the position that a diagnosis of osteoporosis in men under 50 years of age should not be made on the basis of densitometric criteria alone. It also state, for premenopausal women, Z-scores(comparison with age group rather than peak bone mass) rather than T-scores should be used, and the diagnosis of osteoporosis in such women also should not be made on the basis of densitometric criteria alone.(12)

A T-score of -1.0 to -2.5 signifies osteopenia, meaning below-normal bone density without full-blown osteoporosis. This stage of bone loss is the precursor to osteoporosis. Upon measurement completion, a report is generated. The T-score is the number of standard deviations that a patient's BMD is above or below the mean BMD of young, healthy person of the same sex. The Z-score is the number of standard deviations that a patient's BMD is above or below the mean BMD of others of the same age and sex. According to the WHO, a T-score between -1.0 and -2.5 is a sign of osteopenia, and a T-score of -2.5 or below indicates osteoporosis. The report scores of an 80-year-old woman are shown. The Z-score instead of the T-score should be used for healthy premenopausal women, men under age 50, and children. A Z-score less than -2.0 is below the expected range.(13)

Estrogen:is the major sex steroid hormone influencing bone turnover,(14) and maintaining bone mass in men and women. (15) Estrogen has an anabolic action on bone. It indirectly causes a reduction of bone resorption by preventing osteoclastogenesis and can directly inhibit osteoclastfunction. (16) Estrogen acts directly on bone marrow cells, osteoblastline age cells, and osteoclaststo inhibit the production of a number of cytokines including IL-1, IL-6, TNF-α and M-CSF, and these cytokines are associated with estrogen reduction-induced bone loss. (17, 18) Estrogen deficiency, therefore, stimulates osteoclast differentiation, activation and maturation through the significant increase of proinflammatory cytokines including IL-6, granulocyte macrophage colony-stimulating factor and prostaglandin E2, a substance synthesized primarily from arachidonic acid which stimulates the effects of RANK Land inhibits the effects of OPG. (19) Also, increased cytokine production stimulates bone resorption during the bone remodeling process, increases the pool of early osteoclastic precursors in the bonemarrow and stimulates bone loss. (20-22) Several cytokines such as IL-6, PGE2 and TNF-α have a key role in regulating the synthesis of estrogen (23, 24). Bone remodelling activity increases substantially among postmenopausal women and can result in bone loss of approximately 3% annually.(25) Thus, estrogen deficiency after menopause accelerates age-related bone loss, and bone mineral density can decrease significantly by6 years aftermenopause.(26)The colony stimulating factor 1 (CSF1),also known as macrophage colony-stimulating Is a secreted cytokine which influences hematopoietic stem cells to differentiate into macrophages or other related cell types. Eukaryotic cells also produce M-CSF in order to combat intercellular viral infection.(27)It is one of the three experimentally described colony-stimulating factors. M-CSF binds to the colony stimulating factor 1 receptor. It may also be involved in development of the placenta.(28)M-CSF is a hematopoietic growth factor that is involved in the proliferation, differentiation and survival of monocytes, macrophages, and bone marrow progenitor cells. M-CSF affects macrophages and monocytes in several ways, including stimulating increased phagocytic and chemotactic activity, and increased tumor cell cytotoxicity. *(29)*The role of M-CSF is not only restricted to the monocyte-macrophage cell lineage. By interacting with its membrane receptor (CSF1R or M-CSF-R encoded by the c-fms proto-oncogene), M-CSF also modulates the proliferation of earlier hematopoietic progenitors and influence numerous physiological processes involved in immunology, metabolism, fertility and pregnancy. *(30)*M-CSF released by osteoblasts exerts paracrine effects on osteoclasts. M-CSF binds to receptors on osteoclasts inducing differentiation, and ultimately leading to increased plasma calcium levels—through the resorption of bone.(31)High levels of M-csf expression are observed in the endometrial epithelium of the pregnant uterus as well as high levels of its receptor M-CSFR in the placental trophoblast. Studies have shown that activation of trophoblasitc M-CSFR by local high levels of M-CSF is essential for normal embryonic implantation and placental development.(32)More recently, it was discovered that M-csf and its receptor M-CSFR are implicated in the mammary gland during normal development and neoplastic growth.*(33)* Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as CSF2, is a monomeric glycoprotein secreted by macrophages, T cells, mast cells, NK cells, endothelial cells and fibroblasts that functions as a cytokine. The pharmaceutical analogs of naturally occurring CSF2 are called sargramostim and molgramostim. (34) Unlike GM-CSF, which specifically promotes neutrophil proliferation and maturation, CSF2 affects more cell types, especially macrophages and eosinophils. (35) CSF2 is a monomeric glycoprotein that functions as a cytokine ,it is a white blood cell growth factor.(*34)*CSF2 stimulates stem cells to produce granulocytes (neutrophils, eosinophils and basophils)and monocytes. Monocytes exit the circulation and migrate into tissue, whereupon they mature into macrophages and dendritic cells. Thus, it is part of the immune/inflammatory cascade, by which activation of a small number of macrophages can rapidly lead to an increase in their numbers, a process crucial for fighting infection.(36)CSF2 also has some effects on mature cells of the immune system. These include, for example, inhibiting neutrophil migration and causing an alteration of the receptors expressed on the cells surface.(37)CSF2 signals via signal transducer and activator of transcription, STAT5.*(38)*

MATERIAL AND METHODS

According to cross-sectional dual center study was conducted at DEXA unit in radiology department in Al-Sader Teaching Hospital in AL-Najaf Province /Iraq from August 2015 till the end of April 2016 to know the prevalence of Osteoporosis in Iraqi postmenopausal women a total of 154 females from age of 50 year to age of 80year were randomly selected from the patients attending the out patients clinic. Osteoporosis was diagnosed according to WHO criteria.Women were excluded from the study if they had endocrine diseases, environmental factors, diseases with altered activity (like rheumatoid arthritis, cerebrovascular accidents, chronic obstructive pulmonary diseases) or received any anti-osteoporosis treatment, and/or hormone replacement therapy at the time of BMD measurement. A total of 154 women involved in this study with mean

age 63.753 ± 10.2427years .Blood samples had been taken from (122) osteoporosis women and (32) women apparently healthy as a control group .Consent was obtained from the patients' first-degree relatives (mother or father). These patients were also informed that the results of the study would be provided to them as free useful laboratory tests. The patients were diagnosed with osteoporosis as DXEA scan. Diagnosis was established by observing clinical symptoms and conducting hematological . None of these controls was anemic or manifested an evident systemic disease.

Methods A-Assays: Estimation of M-CSF ,GM-CSF and Estrogen quantitatively was performed using a solid-phase enzyme-linked immunosorbent assay (ELISA) supplied by (Bioassay®, china). DEXA measurement and medical history participants with DEXA measurements were classified into different categories of osteoporosis based on their T-score values from their BMD measured by DEXA and their answer to the question regarding fracture . The most important information to check is the correct identification of the patient, his date of birth and also the sex and ethnicity.BMD recorded at the lumbar spine (L1-L4) and left and right femurs, by using dual energy X-ray absorptiometry (DXEA) machine (Dexxum).(Appendix Osteoporosis was diagnosed according to World Health Organization(WHO) guidelines criteria for diagnosis of Osteoporosis.

B-Statistical Analysis: The types of distribution of the variable results were examined using Kolmogorov-Smirnov test. The results of the analysis were calculated by dividing the variables into 2 classes depending on the statistical distribution: normally distributed and non-parametric variables. For normally distributed variables, the results were stated as mean ± standard deviation. Pooled t test was used to compare patients and the control groups. Pearson's correlation coefficient (r) was computed to determine the correlation between parameters. For non-parametric variables that are not normally distributed, the results were expressed as median in addition to mean ± standard deviation. Mann-Whitney U test was utilized to compare the patients and the control groups. Spearman's correlation coefficients (ρ, rho) were calculated to determine the correlation between parameters. Statistical analysis was performed in SPSS version 19.0.1 multilingual program (2010; IBM, USA). A forecasting study was performed using "Regression Forecasting Model" software (Business Spreadsheets, USA).

RESULTS

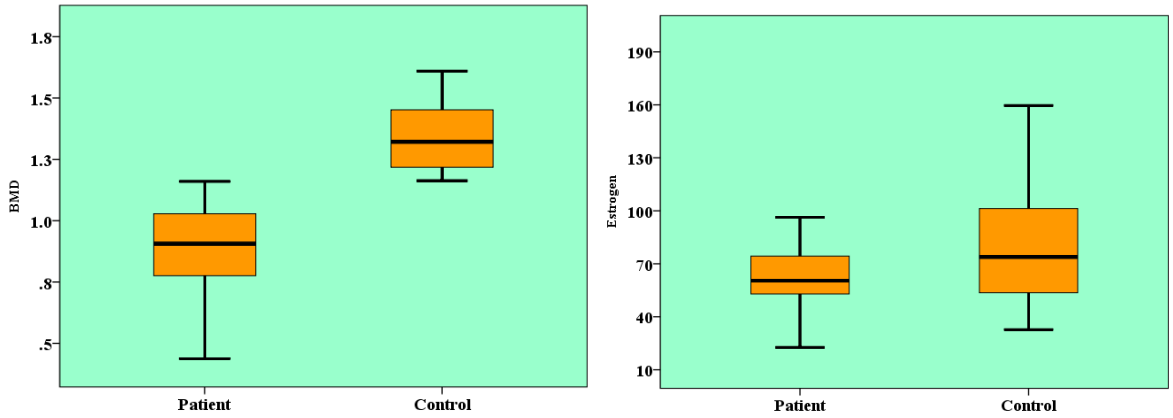
Comparison Between Patients with osteoporosis and Control Group (Table 1 , Figure 1) shows the expected status of osteoporosis associated with postmenopausal women as indicated by a decrease ($p < 0.0001$) in estrogen concentration. Table 1 also shows the significantly high concentration of estrogen and BMD status parameters in patients with osteoporosis compared with the control group The mean of BMD for patients group was (0.902 ± 0.155) and for healthy group was (1.347 ± 0.142) in (mg/cm3) , (P=0.000001).; The mean of Estrogen serum for patients group was (62.322 ± 16.834) and for healthy group was (80.258 ± 36.274) in (ng/ml),(P=0.0001).

In present study there was a no significant in mean serum (M-CSF in(pg/ml)& GM-CSF in(ng/ml)) in postmenopausal Women with osteoporosis was (717.679 ± 223.903), (38.624 ± 17.070) and for healthy group was (691.999 ± 308.896) ,(38.458 ± 40.585) , (P=0.596), (P=0.972) respectively.

Table 1: Comparison between Patients with osteoporosis and Control Group

Parameters	Patients(n=122) (Mean±SD)	Controls(n=32) (Mean±SD)	P value
BMD (mg/cm3)	0.902 ± 0.155	1.347 ± 0.142	0.000001**
M-CSF(pg/ml)	717.679 ± 223.903	691.999 ± 308.896	0.596
GM-CSF(ng/ml)	38.624 ± 17.070	38.458 ± 40.585	0.972
Estrogen(ng/ml)	62.320 ± 16.831	80.250 ± 36.271	0.0001**

ISSN: 0975-8585



**: high significant, *: significant

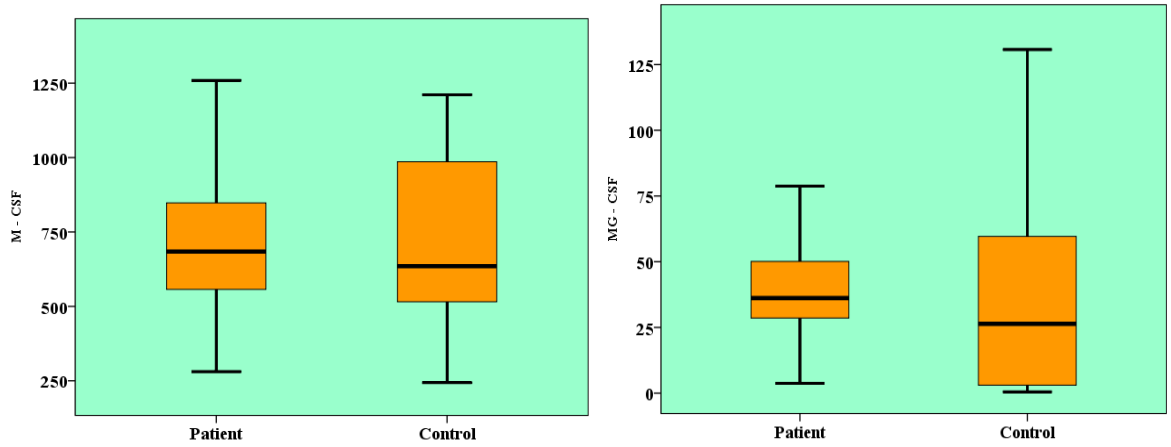


Figure 1: Box Plot Distribution of Studied (BMD, Estrogen, M-CSF and GM-CSF) shows Upper limit, Lower limit, and Median.

Correlation Between M-CSF and GM-CSF Parameters and Estrogen and BMD Status Parameters The results in Table 2 presented the correlation coefficients (p) and p-values for the relationship between M-CSF state parameters and each parameter in osteoporosis patients. The results in Table 2 are very interesting. M-CSF showed a significant positive correlation with GM-CSF (p = 0.0001, r = 0.517) and estrogen (p = 0.00001, r = 0.330), and negative relation between M-CSF and BMD (r = –0.307, p = 0.001). Furthermore, GM-CSF and subsequently , showed a significant correlation with estrogen (r = 0.263, p = 0.0001), and negative relation between GM-CSF and BMD (r = –0.355, p = 0.0001).

Table 2 : Correlation Between M-CSF and GM-CSF Parameters and Estrogen and BMD Status Parameters .

Parameters.	Patients(n=122)		Control(n=32)	
	Correlation Coefficients(r)	P (value)	Correlation Coefficients(r)	P (value)
(M-CSF)VS (GM-CSF)	**0.517****	**0.0001**	**0.330**	**0.065**
(M-CSF)VS (BMD)	**-0.307****	**0.001**	**-0.274**	**0.129**
(GM-CSF)VS (BMD)	**-0.355****	**0.0001**	**0.138**	**0.452**
(M-CSF) VS (Estrogen)	**0.330****	**0.00001**	**-0.030**	**0.869**
(GM-CSF) VS (Estrogen)	**0.362****	**0.00001**	**-0.282**	**0.118**

*** P<0.05, ** P<0.005**

ISSN: 0975-8585

DISCUSSION

Comparison Between Patients with osteoporosis and Control Group. In present study there was a no significant in mean serum (M-CSF in(pg/ml)& GM-CSF in(ng/ml)) in postmenopausal Women with osteoporosis was (717.679 ± 223.903), (38.624 ± 17.070) and for healthy group was (691.999 ± 308.896) ,(38.458 ± 40.585) , (P=0.596), (P=0.972) respectively. This finding were in accordance with results obtained by(39) study.CSF1 related disease states include osteoporosis, destructive arthritis, atherogenesis, glomerulonephritis, Kawasaki disease, and HIV-1 infection, in which monocytes/macrophages and related cell types play a role. For instance, osteoclasts are similar to macrophages and are regulated in part by CSF1. Growth and differentiation signals induced by CSF1 in the initial stages of osteoclast maturation are essential for their subsequent osteoclastic activity in bone.(39)Postmenopausal bone loss results from defective bone remodeling secondary to an uncoupling of bone formation from exuberant osteoclast mediated bone resorption as a consequence of estrogen deficiency. Multinuclear osteoclast formation induced by (CSF1 + RANKL) is completely abrogated by treatment with CSF2.(40) Osteoclast mediated bone loss, in the form of both focal bone erosions and more diffuse articular osteoporosis, is a major unsolved problem .Through our current study of these factors and the narrow possibilities and time needed to study the faces of researchers to study these important factors of other fields used as a treatment for the prevention of complications of osteoporosis and not adopted in the diagnosis of detailed osteoporosis. The results of current study show a significant decrease in mean serum (Estrogen) in postmenopausal Women with osteoporosis was (62.322 ± 16.834) and for healthy group was (80.258 ± 36.274) in(ng/ml),(P=0.0001); This finding were in accordance with results obtained by(55,56,57,58)studied. In our study there was a significant decrease in BMD in(osteoporosis)postmenopausal women(P<0.000001);The present study revealed BMD measured of osteoporosis women is a better test than any other factor measured in the diagnosis of the disease, with incidence of low BMD ,T-score and Z-score in osteoporosis women more than controls women, and this result is supported by other workers: 41,42,43,44,45).Bone mineral density(BMD)showed a significant negatively correlation with (M-CSF, GM-CSF)Serum ,(r =-0.307, P<0.001),(r= -0.355, P<0.0001) respectively, inpatients women only. This is agreement with :39,46,47) ; and BMD is no correlation with (Estrogen)Serum for patients and controls post-menopausal women; This is in accordance with:48-54)studies.

REFERENCES

[1] H. Orimo, T. Nakamura, T. Hosoi et al. (2012) . “Japanese guidelines for prevention and treatment of osteoporosis-executive summary,” Archives of Osteoporosis, vol. 7, no. 1-2, pp. 3–20.

[2] T. W. O'Neill and D. K. Roy(2005) , “How many people develop fractures with what outcome?” Best Practice and Research: Clinical Rheumatology, vol. 19, no. 6, pp. 879–895,.

[3] M. Shiraki, T. Kuroda, and S. Tanaka, (2011) . “Established osteoporosis associated with high mortality after adjustment for age and co-mobidities in postmenopausal Japanese women,” Internal Medicine, vol. 50, no. 5, pp. 397–404,.

[4] Link TM. Axial and peripheral QCT. Guglielmi G, ed. *Osteoporosis and Bone Densitometry Measurements*. New York, NY: Springer Heidelberg; 2013. 123-32.

[5] Yang L, Palermo L, Black DM, Eastell R. Prediction of incident hip fracture with the estimated femoral strength by finite element analysis of DXA Scans in the study of osteoporotic fractures. *J Bone Miner Res*. 2014 Dec. 29(12):2594-600.

[6] Irish Osteoporosis Society 2013.

[7] Chun, K. J. (2011, May). Bone densitometry. In Seminars in nuclear medicine(Vol. 41, No. 3, pp. 220-228). WB Saunders.

[8] Bow, C. H.; Cheung, E.; Cheung, C. L.; Xiao, S. M.; Loong, C.; Soong, C.; Tan, K. C.; Luckey, M. M.; Cauley, J. A.; Fujiwara, S. and Kung, A. W. (2012). Ethnic difference of clinical vertebral fracture risk. OsteoporosInt, 23(3): 879-885.

[9] Henwood, MJ; Binkovitz, L (2009) . "Update on pediatric bone health".The Journal of the American Osteopathic Association 109 (1): 5–12.

[10] Erlandson, K. M., Guaraldi, G., &Falutz, J. (2016). More than osteoporosis: age-specific issues in bone health. Current Opinion in HIV and AIDS, 11(3), 343-350.

[11] http://www.who.int/chp/topics/Osteoporosis.pdf. Accessed February 23, 2015.

[12] Leib ES, Lewiecki EM, Binkley N, et al.,(2004), International Society for Clinical Densitometry. Official positions of the International Society for Clinical Densitometry. J ClinDensitom. Spring;7:1-6.Sonia , et al 2016
[13] Almeida, M., Laurent, M. R., Dubois, V., Claessens, F., O'Brien, C. A., Bouillon, R., ... &Manolagas, S. C. (2017). Estrogens and Androgens in Skeletal Physiology and Pathophysiology. Physiological Reviews, 97(1), 135-187
[14] Syed,F.,&Khosla,S.(2005).Mechanismsofsexsteroideffectsonbone.Biochemicaland Biophysical Research Communications, 328(3),688-696.
[15] Bellido, T. (2014). Osteocyte-driven bone remodeling. Calcified tissue international, 94(1), 25-34
[16] Syed,F.A.,Mödder,U.I.L.,Roforth,M.,Hensen,I.,Fraser,D.G.,Peterson,J.M.,...Khosla,S. (2010).Effectsofchronicestrogentreatmentonmodulatingage-relatedbonelossin female mice. Journal of Bone and Mineral Research, 25(11),2438-2446.
[17] Lorenzo,J., Horowitz,M.,&Choi, Y. (2008).Osteoimmunology:interactionsoftheboneand immune system. Endocrine reviews, 29(4),403-440.
[18] Clowes,J.A.,Riggs,B.L.,&Khosla,S.(2005).Theroleoftheimmunesysteminthe pathophysiology of osteoporosis. Immunological reviews, 208(1),207-227.
[19] Horowitz,M.C.(1993).Cytokinesandestrogen inbone:anti-osteoporoticeffects. Science,260(5108),626.
[20] Clowes,J.A.,Riggs,B.L.,&Khosla,S.(2005).Theroleoftheimmunesysteminthe pathophysiology of osteoporosis. Immunological reviews, 208(1),207-227.
[21] Pfeilschifter,J.,Köditz,R.,Pfohl,M.,&Schatz,H.(2002).Changesinproinflammatorycytokine activity after menopause. Endocrine reviews, 23(1),90-119.
[22] Purohit,A.,Newman,S.P., &Reed,M. J. (2002).Theroleofcytokines in regulatingestrogensynthesis:implicationsfortheetiologyofbreast cancer.BreastCancerResearch,4(2),65-69.
[23] Reed, M., &Purohit, A. (2002). Aromatase regulation and breast cancer. Clinical Endocrinology,54(5),563-571.
[24] Hoeppner LH, Secreto FJ, Westendorf JJ: Wntsignaling as a therapeutic target for bone diseases. Expert OpinTher Targets 2009;13:485-496.
[25] Karlsson, M. K., Ahlborg, H. G., Svejme, O., Nilsson, J. Å., &Rosengren, B. E. (2016). An Increase in Forearm Cortical Bone Size After Menopause May Influence the Estimated Bone Mineral Loss—A 28-Year Prospective Observational Study. Journal of Clinical Densitometry, 19(2), 174-179
[26] Hochrein, Hubertus, and Meredith O'keeffe. "Induction of dendritic cell development with macrophage-colony stimulating factor (M-CSF)." U.S. Patent No. 8,445,275. 21 May 2013
[27] Ushach, I., &Zlotnik, A. (2016). Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. Journal of Leukocyte Biology, 100(3), 481-489
[28] Aubin,J.E.,Lian,J.B.,Stein,G.S.(2006).Boneformation:maturationandfunctionalactivities ofosteoblastlineagecell.InM.J.Favus(Ed.),Primeronthemetabolicbonediseasesand disorders of mineral metabolism (pp. 20-27). Washington, DC: American Society forBone and MineralResearch
[29] Jang MH, Herber DM, Jiang X, Nandi S, Dai XM, Zeller G, Stanley ER, Kelley VR.Distinct in vivo roles of colony-stimulating factor-1 isoforms in renal inflammation.JImmunol. 2006 Sep 15;177(6):4055-63.
[30] Stanley F.J., E.R. CSF-1 regulation of the wandering macrophage: complexity in action Trends Cell Biol., 14 (2004), pp. 628–638
[31] Hienz, S. A., Paliwal, S., &Ivanovski, S. (2015). Mechanisms of bone resorption in periodontitis. Journal of immunology research, 2015
[32] Gow, D. J. (2013). Role of macrophage colony stimulating factor-1 (CSF-1) in postnatal growth.
[33] Steidl, S., Hamilton, J. A., & Cook, A. D. (2016). U.S. Patent No. 9,243,066. Washington, DC: U.S. Patent and Trademark Office.
[34] Francisco-Cruz, A., Aguilar-Santelises, M., Ramos-Espinosa, O., Mata-Espinosa, D., Marquina-Castillo, B., Barrios-Payan, J., & Hernandez-Pando, R. (2014). Granulocyte–macrophage colony-stimulating factor: not just another haematopoietic growth factor. Medical oncology, 31(1), 774
[35] Delves, P. J., Martin, S. J., Burton, D. R., &Roitt, I. M. (2017). Essential immunology. John Wiley & Sons,49-65.
[36] *Hansen PJ, Dobbs KB, Denicol AC (Sep 2014). "Programming of the preimplantation embryo by the embryokine colony stimulating factor 2". Animal Reproduction Science. 149 (1-2): 59 66.* doi:10.1016/j.anireprosci.2014.05.017. PMID 24954585.

[37] Gasson JC (Mar 1991). "Molecular physiology of granulocyte-macrophage colony-stimulating factor". Blood. 77 (6): 1131–45. PMID 2001448.
[38] *Voehringer D (Oct 2012). "Basophil modulation by cytokine instruction". European Journal of Immunology. 42 (10): 2544–50.* doi:10.1002/eji.201142318. PMID 23042651.
[39] Breuil, V., Ticchioni, M., Testa, J., Roux, C. H., Ferrari, P., Breittmayer, J. P., ... &Euller-Ziegler, L. (2010). Immune changes in post-menopausal osteoporosis: the Immunos study. Osteoporosis International, 21(5), 805-814
[40] Miyamoto, H., Suzuki, T., Miyauchi, Y., Iwasaki, R., Kobayashi, T., Sato, Y.,&Hao, W. (2012). Osteoclast stimulatory transmembrane protein and dendritic cell–specific transmembrane protein cooperatively modulate cell–cell fusion to form osteoclasts and foreign body giant cells. Journal of Bone and Mineral Research, 27(6), 1289-1297
[41] Blake, G. M., & Fogelman, I. (2007). The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. Postgraduate medical journal, 83(982), 509-517
[42] Asciak, R., Attard, C., Casha, R., Barbara, P., &Coleiro, B. (2013). Audit on follow-up of patients with primary Osteoporosis
[43] Siddapur, P. R., Patil, A. B., &Borde, V. S. (2015). Comparison of bone mineral density, T-scores and serum zinc between diabetic and non diabetic postmenopausal women with osteoporosis. Journal of laboratory physicians, 7(1), 43
[44] Sheu, A., & Diamond, T. (2016). Diagnostic tests: Bone mineral density: testing for osteoporosis. Australian prescriber, 39(2), 35
[45] Ward, MDa , Catherine C. Roberts, MDb , Jenny T. Bencardino, MDc , Erin Arnold, MDd , Steven J. Baccei, MD ,et al., ACR Appropriateness Criteria Osteoporosis and Bone Mineral Density.AmCollRadiol 2017;14:S189-S202.
[46] Seifert-Klauss, V., & Prior, J. C. (2010). Progesterone and bone: actions promoting bone health in women. Journal of osteoporosis, 2010
[47] Wu, Dongmei; Liu, Peicheng; Xu, Zuolan; Gu, Lijiang; Wang, Chunyan; Li, Ting; Li, Jun Study of the correlation between the serum level of vitamin d and osteoporosis in the elderly people in urumqi ,ZhongguoGuzhiShusongZazhi (2015), 21(2), 183-187.
[48] Worley, Richard J.Age, estrogen, and bone density,From Clinical Obstetrics and Gynecology (1981), 24(1), 203-18.Adams, Judith E. "Dual-energy X-ray absorptiometry." Radiology of osteoporosis. Springer Berlin Heidelberg, 2003. 87-100.
[49] Korczowska, Izabela; Olewicz-Gawlik, Anna; Hrycaj, Pawel; Lacki, Jan. The effect of long-term glucocorticoids on bone metabolism in systemic lupus erythematosus patients: The prevalence of its anti-inflammatory action upon bone resorption, Yale Journal of Biology and Medicine (2003), 76(2), 45-54.
[50] Shang, Hua; Yin, Yousheng; Li, Xiaoli; He, Yong; Qiu, Weijia; Deng, Yilan; Li, Xiaohong; Li, Kanghui, Effect of estrogen on pathogenesis of osteoporosis in women with maintenance hemodialysis ,From ZhonghuaNeifenmiDaixieZazhi (2010), 26(2), 108-110.
[51] Yoldemir, Tevfik; Erenus, Mithat; Durmusoglu, Fatih .The impact of serum FSH and estradiol on postmenopausal osteoporosis related to time since menopause,From Gynecological Endocrinology (2012), 28(11), 884-888.
[52] Chen, Chengwang; Pan, Xiaoyun; Xue, Enxing; Wen, Hong, Comparison of effectiveness of parathyroid hormone, calcitonin and alendronate in treatment of established osteoporosis for postmenopausal women.From Wenzhou YixueyuanXuebao (2013), 43(10), 670-673.
[53] Liu, Haiyan; Wang, Huaxin; Li, Weina; Ji, Jing . Application of detection of parathyroid hormone and vitamin D in diagnosis of osteoporosis From HebeiYiyao (2014), 36(24), 3799-3802.
[54] Song, Hong; Huang, Hua; Wang, Wei; Song, Bin; Wang, Rong;Peng, Hanyuan; Cui, Jing; Hao, Chuan .Study on the effect of different gender and age on bone metabolic indexes, serum osteoprotegerin and bone mineral density in primary osteoporosis,FromZhongguoGuzhiShusongZazhi (2015), 21(10), 1161-1164.
[55] Beck, M. M.; Hansen, K. K. Role of estrogen in avian osteoporosis, Poultry Science (2004), 83(2), 200-206.
[56] Cauley, Jane A. "Estrogen and bone health in men and women." Steroids 99 (2015): 11-15.
[57] Lindsay, R. (2015). Estrogens, progestins, serms, and osteoporosis. In Nutrition and Bone Health (pp. 57-63). Springer New York.
[58] Lerner, U. H. (2016). Bone remodeling in post-menopausal osteoporosis. Journal of dental research.