ORIGINAL ARTICLE

Study of Association of Serum Concentration of Minerals with Osteoporosis in Postmenopausal Women

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ABSTRACT

Background: Complete radiological assessment was made that aid in diagnosis of osteoporosis. One of the vital tools in assessment of osteoporosis is via Dual-energy X-ray absorptiometry (DEXA) for screening women that are >45 of age and then final assessment is made on basis of T-scores obtained.
Aim: To estimate and detect serum levels of calcium, phosphorus, magnesium, iron and zinc levels in individuals that are under examination and then comparison of these individuals with individuals in healthy control group.
Place and time of study: DHQ Teaching Hospital Sargodha from January 2019 to March 2020
Methods: Serum calcium, magnesium, iron, zinc and phosphorous were measured and then comparison was made among the candidates with osteoporosis (120) and sound healthy individuals (30). Final assessment of patient with osteoporosis was made by DEXA check.
Results A Comparison was made among postmenopausal women belonging to osteoporosis group and control group and it was found that there was a serious decline in serum levels of calcium in case of postmenopausal women belonging to osteoporosis group. Moreover, there was decline in serum magnesium, phosphorus, zinc and iron levels in candidates with osteoporosis when compared with healthy individuals. There was significant decrease in T-scores observed in postmenopausal women belonging to osteoporosis group when comparison was made with individuals in healthy group.
Conclusion In case of postmenopausal women having osteoporosis there was either low or normal serum levels of calcium, magnesium phosphorus, zinc and iron levels when comparison was made with the controls group. In order to detect osteoporosis in postmenopausal women DEXA is the method of choice with the help of T-scores.
Keywords osteoporosis, calcium, phosphorus, magnesium, iron, zinc, T-scores, DEXA

INTRODUCTION

One of the most common disorders of bone is Osteoporosis in which there is declination of bone density and aggravation of trabecular bone auxiliary respectability occurs. There is thinning of Cortical bone observed as well as it also becomes fragile. All these harmful disastrous consequences make bones weak and easily prone to factures. One of the characteristics of osteoporosis is that there is reduction of BMD (Bone Mineral density) and at the same time increased chances of bony factures[1]. As a result of these facture, patients suffers from mental anxiety, raise horridness, mortality and stature changes along with hospitalization for extended period of time[2,3]. BMD measurement gives us the diagnosis of osteoporosis[4]. BMD can be effectively calculated by the aid of the dual-energy X-ray absorptiometry (DEXA). Final diagnosis of osteoporosis is based on patient's history, physical examination and finally lab tests. Examinations and test are conducted depending upon tumor examination with metastasis deep down, Cushing's infection various myeloma, and other aspects of patient history. The critical criteria for diagnosis of osteoporosis include biochemical markers, estimation of BMD and radiographic assessment[5]. For analysis of damage that has been done by osteoporosis, density testing is used. To check and observe other ligaments and bone, X-beam films are in practice. It is possible to analyze the thin bones on X-ray film, but still bone thickness testing is more accurate. Early diagnosis of osteoporosis is possible as osteoporosis can be detected after having cracks in bone from minor injuries with the aid of BMD estimation along with DEXA check, or by observing fault on X-ray film[6,7]. BMD test, helps us to distinguish the bone mass measurements in entire body and also helps us to calculate bone density . According to some researches, at any anatomical site, the knowledge about bone mineral density is equally important for calculating the danger of crack[14-17]. DXEA is regarded as the best and high quality source for osteoporosis diagnosis. When BMD value is equal or less than 2.5 (that is regarded as standard deviations) of youth 30-40 year old, osteoporosis is been suspected and analyzed[12]. This score is regarded as a T-score. The bone density seems to be decreased with the increase in age so more people are diagnosed with osteoporosis as they grow older[4,6] the diagnostic criteria as per by World Health Organization has following diagnostic guidelines[15,21]. According to the International Society for Clinical Densitometry, osteoporosis in women whose age is below 50 should not to be diagnosed with this disease depending only on densitometry criteria. Moreover, it also states that for premenopausal women T-scores should be utilized and diagnosis of osteoporosis only on densitometry criteria should not be made[27]. If the score lies 2.5 or below then

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this shows presence of osteoporosis[25]. For optimal human skeletal growth, minerals are vital foe proper nourishment and development . In addition to minerals, there are micro building components found in bone that plays an important role in bone metabolism as well as in bone turnover. It is still not clear about the role of trace elements in osteoporosis[31, 33].

MATERIAL AND METHODS

This research was carried at DEXA unit located at DHQ Teaching Hospital Sargodha in January 2019 to March 2020. About, 150 female candidates having age ranged from 45–70 years with osteoporosis were selected for this random study. The diagnosis of osteoporosis in these women was made according to criteria set by World Health Organization (WHO). All those women having diseases like endocrine disturbances, diseases due to environmental factors or diseases with altered activity such as cerebrovascular mischances, incessant obstructive aspiratory sicknesses and rheumatoid arthritis were excluded. More over women, who responded aggressively to osteoporosis treatment, or having hormone substitution treatment were also excluded. This cross sectional study included 150 women having mean age 60.66 ± 11.23 years. Blood samples were collected from both women belonging to osteoporosis group (120 females) and control group (30 women). From all the females' first-degree relatives, a written consent was taken. All the laboratory tests of these patients were done free of cost. Candidates having osteoporosis were diagnosed on basis of DEXA scan. The final diagnosis and assessment was based on presence of clinical symptom in candidates with osteoporosis as well as from blood tests. Candidates of control group had no reporting of anemic or system disease.

Methods Assays with the aid of calorimetric method, the calculation of phosphorus, magnesium, zinc and iron, was performed quantitatively by using auto biochemistry analyzer, units calcium provided by UOS. All the members who estimated DEXA categorized DEXA estimations into different grades of osteoporosis with respect to T-score as determined by DEXA and their reaction to the inquiry with respect to breaking. In order to determine this fact, patient's date of birth, sex and ethnic play an important role in accurate determination and detection. T-scores recorded at the lumbar spine (L1– L4), T score was calculated with the help of DEXA machine (Dexum). The diagnosis of Osteoporosis was done based on the criteria set by a World Health Organization (WHO).

Statistical analysis: The data obtained from present study was analyzed statistically as Mean ± standard errors. The GraphPad Prism was used to calculate descriptive statistics, correlation coefficients, and P-value. The *t*-test was used to compare between two groups and then final assessment was made by SPSS 22.0. *P*-value < 0.05 was statistically significant.

RESULTS

The comparison was made between candidates having osteoporosis and candidates who were healthy. From the table 1 observation, there was significant differences were seen in osteoporosis group when compared with healthy group, as value of ($p < 0.05$) was significantly decreased in case of minerals like in serum, magnesium, calcium, phosphorus, zinc, and iron. In case of T-scores comparison it was observed that osteoporosis group candidates have serious decline in levels of serum calcium in postmenopausal women as compared to candidates in healthy control group as shown in Fig. 2. Moreover, it was also cleared that Serum phosphorus was also significantly decline in case of postmenopausal candidates in osteoporosis group as compared to candidates in healthy group as shown in Fig.2. It was also observed that there was decline in serum magnesium levels in post-menopausal osteoporosis group member as compared to healthy control group individuals as shown fig.2. According to fig.2, there was also decline in levels of serum iron and serum zinc in postmenopausal female candidates of osteoporosis group as compared to individuals in healthy group. According to fig 2, T score also scored very less in post-menopausal women of osteoporosis group when compared with healthy group individuals. From our research, serum calcium for patients group was 7.99 ± 0.09 and in case of healthy group individuals was 11.02 ± 0.12 mg/dL, ($P < 0.0001$). The mean value of serum phosphorus for patients group was 1.71±0.08 where as in case of healthy group was 4.01 ± 0.15 mg/dL, ($P < 0.0001$). The mean value of serum magnesium for patients group was 2.00±0.07 and for controls group was 2.36 ± 0.10 mg/dL, ($P = 0.0114$). The mean of serum iron for patients group was 89.00± .72 and in case of controls group was 138.10 ± 3.44 mg/dL, ($P < 0.0001$) value of serum zinc for patients group was 90.90±2.52 and in case of controls group was 104.06± 2.99 mg/dL, ($P=0.0012$); and the mean of T-scores for osteoporosis group was −3.913±0.301 and in case of for controls group its value was recorded as 1.92±0.17 ($P < 0.0001$).

Fig.1: The main elements of human body composition

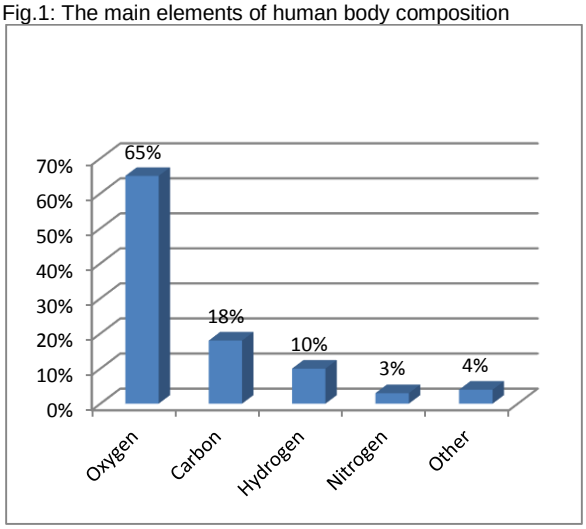
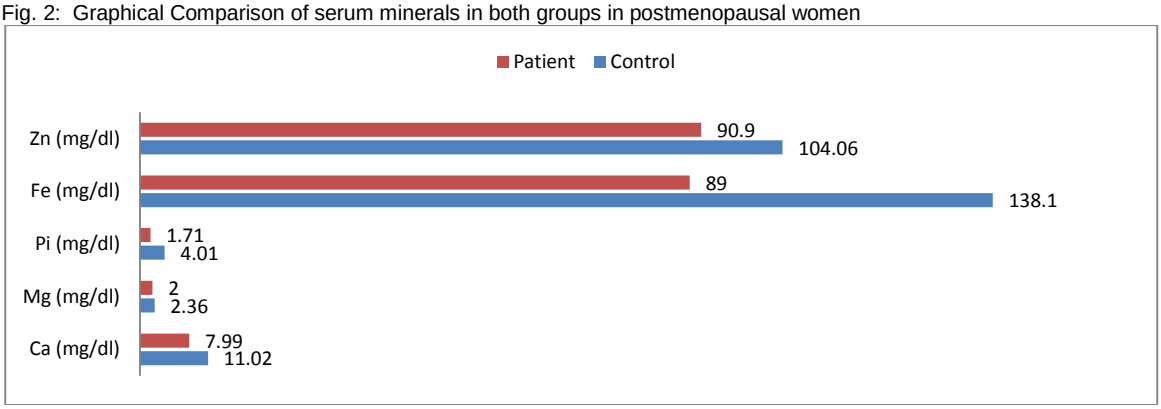


Table 1: Comparison of serum minerals in both groups in postmenopausal women

Minerals	Control (n=30) (Mean ±S.E.)	Patients(n=120) (Mean ± S.E.)	p value
Ca (mg/dl)	11.02 ± 0.12	7.99 ± 0.09	<0.0001
Mg (mg/dl)	2.36 ± 0.10	2.00 ± 0.07	<0.0114
Pi (mg/dl)	4.01 ± 0.15	1.71 ± 0.08	<0.0001
Fe (mg/dl)	138.10 ± 3.44	89.00 ± 2.72	<0.0001
Zn (mg/dl)	104.06 ± 2.99	90.90 ± 2.52	<0.0012

Fig. 2: Graphical Comparison of serum minerals in both groups in postmenopausal women



DISCUSSION

For a healthy living and wellbeing optimum intake of minerals like zinc, magnesium, calcium, phosphorus and iron are essential for proper growth and development. All these minerals play a vital role in bone heath and essential for metabolism of bone and its nourishment. From many case researches it is clear that minerals like zinc, calcium, magnesium, phosphors and iron are vital for synthesis of organic bone[24]

As clearly depicted in Table 1that serum magnesium, phosphorus, calcium, magnesium and zinc are significantly decreased in case of postmenopausal women with osteoporosis ($P < 0.05$) as compared to individuals belonging to control group

Serum calcium: On the basis of results obtained from both groups, it was observed that serum calcium level was same in post-menopausal candidates of osteoporosis group as well as in healthy group of individuals .The same serum calcium in both groups was due to the fact that in osteoporosis there is decline in the bone mineralization as a whole without effecting the ratio of bone mineral to the organic matrix. So, as a whole overall there is loss of bone. The levels of serum calcium in the candidates from osteoporosis group was mean 7.99±0.09mg/dl, while in case of healthy post-menopausal women was 11.02±0.12mg/dl, as depicted in Fig. 2, where as normal range of serum calcium is 8.1–10.6mg/dl. So, it was reported that mean serum calcium level was normal in candidates belonging to both the groups. Many researches also proof similar results as proved our case study that serum calcium plays insignificant role in detection of osteoporosis due to the fact that their outcomes were inside typical range[17,18,20-23].

Both calcium and vitamin D play an important role in calcium metabolism. Calcium level as well as convergence of flowing ionized calcium will decline if there is insufficient ingestion of calcium or vitamin D by an individual. When all such consequences rises, there is increase in release of parathyroid hormone (PTH) that subsequently fortify PTH level due to decline in calcium and vitamin D intake. As a result of all these events, there is a big loss and destruction of bone leading increase its fragility and easily prone to cracks and factures.

Serum magnesium: In case of all the patients' belonging to osteoporosis group, lower serum magnesium level was observed as compared to the control group of health postmenopausal women. In case of candidates of osteoporosis group, the mean value of serum magnesium in post-menopausal women was2.00 ± 0.07) mg/dl, where as in case of healthy individuals , the observed value of serum magnesium was 2.36 ± 0.10mg/dl which is clearly depicted in Fig. 2 whereas 1.8–2.3 mg/dl is regarded as normal range for serum magnesium . Other similar case research reported that the mean serum magnesium in osteoporosis group was lower than 2.00 mg/dl observed in case of 12% osteoporosis group candidates, and this is regarded as significant value. Such results clearly aid researchers in detecting osteoporosis[24]. Moreover, other similar studies also reported the similar results in osteoporosis post-menopausal women. The mean value obtained after research was a bit more than 1.7 mg/dl for patients gathering ($P = 0.0114$). It was observed that about 78% of the patients having magnesium levels lower than 1.7mg/dl. Due to these results, researchers should work and exam more and more facts about serum magnesium and its relationship with the parathyroid hormone levels and kidneys. Since magnesium is sufficient intracellularly, so fault might be preset in the vehicle of magnesium in the favors of more intracellular levels[24,25].

According to search result of Mishra et al. (2015) level of serum magnesium was much significantly less ($P < 0.0001$) in case of osteoporosis post- menopausal women compared with women having sound health[26].

Bone is the main storage center of magnesium that is responsible for storing 60–65% of the total body's magnesium. Many studies graded BMD as best test for diagnostic purpose but still researchers are not clear to figure properly the role of magnesium in osteoporosis bony factures[24]. Although magnesium deficiency is reported usually more as compared to magnesium excess still some studies suggested that both low or high levels of magnesium than normal values are detrimental to the health of bone[26]. Proper homeostasis of magnesium is very important for bony health and its nourishment. According to various test and epidemiological researches both either lower or higher than normal serum magnesium levels have some effects to boney structure. Decrease in serum magnesium levels aggravates process of osteoporosis by impacting its effect on a precious stone arrangement and cells in bone and in a roundabout way by influencing the release as well as action of parathyroid hormone and by promoting second rate irritation[25]. So maintenance of magnesium is very much important with respect to

maintaining and keeping up bone integrity[26]. Decline in serum magnesium levels cause modification in the structure of apatite precious stones. So, it is for sure that trabecular bone in post-menopausal women with osteoporosis and decline serum magnesium levels have bigger sorted out precious stones when compared with ladies in control group. Due to these bigger gems, bone lost its ability to bear typical load. Moreover, it was found that decline in serum levels of magnesium is related with decline in PTH levels in body and ultimately decline of vitamin D[24,26].

Serum phosphorus: From case research study, it was found that serum phosphorus was same in both post-menopausal women candidates belonging to osteoporosis group and control group due to the fact that osteoporosis effects the mineralization of bone without affecting the proportion of bone mineral to an organic matrix. As a result of these consequences, there is decree in bone measurement was observed over all. In case of candidates of osteoporosis group, the mean serum phosphorus observed was 1.71±0.08mg/dl, where as in case of control group its value was 4.01±0.15 mg/dl, as depicted in Fig. 2, and the normal range observed value of serum phosphors is 2.2–5mg/dl. By measuring the mean values of both groups with normal value, it was clear that both groups have serum phosphors within normal range. Just as the case research of our study, few researchers also found that mean serum phosphorus is not a noteworthy criteria for osteoporosis analysis and detection due to the fact that its outcomes were within an ordinary range[17,18,27].

According to the case research of Mishra et al. (2015), no critical distinction of mean serum phosphorus level were found in the group of post-menopausal women[25]. Severe inadequacy of phosphorus is linked with calcium arrival from the human skeleton, which result in lowering of bone mineralization. Takeda et al (2012) have been accounted for inadequate generation of 1,25-hydroxyvitamin D, increase in serum PTH[33] and from intestine, decrease in calcium retention[28-31].

Serum iron: The mean serum iron recorded in candidates of osteoporosis group in was 89.00 ± 2.72 mg/dl, where as in case of group of healthy individuals the mean serum iron was 138.10 ± 3.44mg/dl as depicted in Fig. 2. The results of these studies are in accordance of other case studies[31-34].

Iron plays a vital role in transportation of oxygen and is a part of various enzymatic activities in body that are involved in vitamin D absorption and collagen union. So inadequate ingestion of this iron leads to bony destruction leading to its resorption and ultimately increases the hazards of osteoporosis[35]. Iron serve as a co factor for enzymes that plays an important role in synthesis of collagen[36] and it has an important role in vitamin D activation and aids in absorption of calcium[27]. In case of osteoporosis, there is low serum iron as well as lower BMD in case of women undergoing post menopause[37]. Moreover, too much iron stores or ingestion is poisonous to bone cells and cause bone maladies[36,38,39]. The iron over loading in body aggravates oxidative anxiety in individuals body, which leads to destructive changes that ultimately leads to bone resorption. *In vitro*, iron itself affect osteoblastic activity[38] as in case of hemochromatosis patients the decrease in markers of bone formation observed is observed[40]. Ultimately, chelators like deferoxamine hold back the multiplication and replication of osteoblast, fibroblast, osteoblast collagen arrangement and aggravate apoptosis of osteoblast. Iron is considered as vital for cell metabolism and its development so it is not surprising to say iron deficiency aggravates bone digestion and resorption. Plack may have more critical well-being repercussions and a higher effect on open health[41], carried an examination in osteoblast cells found in human body to know the impact of low and high values of serum iron on osteoblastic activity. Their examination found that over loading of iron stores cause decline in osteoblast movement in a fixation subordinate way; whereas just a little iron deficiency advances action of osteoblast and serious lower levels slower down the process of osteogenesis. Many case studies reported clearly the co relationship of dietary iron limitation and bone well-being and it was observed that decrease in iron stores of body significantly influence the bone health and ultimately BMD. In a few investigations, decrease in bone development markers or markers that aggravate bone resorption were significantly found[34]. These parameters like bone resorption and decreased bone development can be recovered by ingestion of a typical or high-press diet[39]. In postmenopausal ladies with no history of osteoporosis, dietary iron and BMD were found to be co related with each other[44]. From other case report, it was found that increased serum transferrin levels and decreased ferritin levels (yet not significant) in candidates with osteoporosis diverged from controls[32]. Therefore, the hormonal circumstance assumes a part tweaking the impacts of iron on bone health[35]. A relationship between digestion of iron and bone was first resolved from examination of patient clinically that depict a higher occurrence osteoporosis and cracks in individuals with scatters of iron digestion, for example, as observed in case of sickle cell disease, inherited hemochromatosis and thalassemia[45].

Serum zinc: In case of candidates of osteoporosis group the results were 90.90 ± 2.52 mg/dl for serum zinc , where as in case of control group candidates, it was 104.06 ± 2.99 mg/dl, as clearly depicted in (Fig. 2). All these results showed similarity[24,40] with other researches regarding this. Zinc is regarded as an important element and act as a catalyst in osteoblasts that play a vital role for collagen union and other products[47]. From other researchers, it was clear that two elements zinc and magnesium play an important basic role in bony framework formation[16] and insufficiency of magnesium may result in declining bone development, keeping the ideal gem arrangement and influence PTH in a negative manner[28]. Moreover, in case of calcium digestion, zinc plays an important role in aggregation of physiological movement of vitamin D[32].

Mutlu et al (2007) by his case researches showed that there is decline of serum magnesium and zinc levels in case of candidates of osteoporosis group as compared to candidates belonging to healthy and sound group[16].

Sadeghi, et al. (2014) in Iran reported that in case of patients with boney factures, there is huge decline in mean serum zinc was noteworthy from normal range and zinc supplementation increases the level of serum zinc significantly and have positive influence on callus formation[36]. Although from different researches it was recommended that lower serum zinc might be due to lower zinc ingestion[18].

CONCLUSIONS

The levels of serum calcium, phosphorus, iron, zinc and magnesium in postmenopausal women having osteoporosis, have a significant relationship with the serum results of controls group individuals and the results were either low or in normal range when compared. These parameters are very essential in detection and diagnosis of osteoporosis. Final diagnosis of osteoporosis by using DEXA scan is the method of choice with the help of T-scores calculations.

Recommendations: All the women reaching to post-menopausal state should undergo DEXA scanning in order to save herself from future bony factures due to decreased bone mineral density, bone fragility and weakness. The use of DEXA scan during treatment of osteoporosis especially during follow up also reduces the bony factures risks to a great extent. In occurrence of osteoporosis, genetic factor plays an important role that's why anyone having Family history of osteoporosis should undergo scan testing often in order to save him from deleterious effects of this disease. More and more researchers are needed on this topic of osteoporosis so that it can cover each and every aspect of this disease. It is therefore need of time to make a group of professionals who can deal with this disease and treat people as take it as a public health problem.

Conflict of Interest: None

REFERENCES

1. Cronin, H., O'regan, C., Finucane, C., Kearney, P., & Kenny, R. A Health and aging: development of the Irish Longitudinal Study on Ageing health assessment. Journal of the American Geriatrics Society, 2013, 61: S269-S278
2. Chun KJ. Bone densitometry. In Seminarsin nuclear medicine (Vol. 41, No. 3,pp. 220–228). Philadelphia, PA:WB Saunders.
3. Bow CH, Cheung E,Cheung CL, Xiao SM, Loong C,Soong C,et al. Ethnic difference of clinical vertebral fracture risk. Osteoporos Int. 2012;23:879–885.
4. Henwood MJ, Binkovitz L. Update on pediatric bone health. J Am Osteopathic Assoc.2009;109:5–12.
5. Erlandson KM, GuaraldiG, Falutz J. More than osteoporosis: age-specific issuesinbonehealth.CurrOpinHIVAIDS.2016;11:343–350.
6. http://www.who.int/chp/topics/Osteoporosis.pdf. Accessed February 23,2015.
7. Leib ES, Lewiecki EM, Binkley N. Official positions of the International Society for Clinical Densitometry. J Clin Densitom. 2004;7:1–6.
8. Almeida M, Laurent MR, Dubois V, Claessens F,O'Brien CA, Bouillon R. Estrogens and androgens in skeletal physiology and pathophysiology. physiological Rev. 2017;97:135–187.
9. Aaseth J, Boivin G, Andersen O. Osteoporosis and trace elements an overview. J Trace Elm Med Biol. 2012;26:149–152
10. Orimo H, Nakamura T, Hosoi T, Iki M, Uenishi K, Endo N, et al. Japanese guidelines for prevention and treatment of osteoporosis-executive summary. Arch Osteoporos. 2012;7:3–20.
11. O'Neill TW, Roy DK. How many people develop fractures with what outcome? Best Pract Res Clin Rheumatol. 2005;19:879–895.
12. Shiraki M, Kuroda T, Tanaka S. Established osteoporosis associated with high mortality after adjustment for age and co-mobidities in postmenopausal Japanese women. Int Med. 2011;50:397–404.
13. Link TM. Axial and peripheral QCT. Guglielmi G, ed. Osteoporosis and Bone Densitometry Measurements. New York, NY: Springer Heidelberg, 2013;123–132.
14. YangL, 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;29: 2594–2600
15. TaitAH,Raubenheimer D, Stockin KA, Merriman M, Machovsky Capuska GE. Nutritional geometry and macronutrient variation in the diets of gannets: the challenges in marine field studies. Marine Biol. 2014;161:2791–2801.
16. Mutlu M, Argun M, Kilic E, Saraymen R, Yazar S. Magnesium, zinc and copper status in osteoporotic, osteopenic and normal post-menopausal women. J Int Med Res. 2007;35:692–695.
17. Al-Maatouq MA, El-Desouki MI, Othman SA, Mattar EH, Babay ZA, Addar M. Prevalence of osteoporosis among postmenopausal females with diabetes mellitus. Saudi Med J.2004;25:1423–1427.
18. Omrani GR, Masoompour SM, Sadegholvaad A, Larijani B. Effect of menopause and renal function on vitamin D status in Iranian women. East Mediterr Health J. 2006;12:188–195.
19. Shakoor S, Ilyas F, Abbas N, Mirza MA, Arif S. Prevalence of osteoporosis in relation to serum calcium and phosphorus in aging women. JGlob Innov Agric Soc Sci. 2014;2:70–75.
20. Lavanya Y, Srikanth S, Satya CM. Vitamin D, serum calcium and bone mineral density in pre and postmenopausal women—a pilot study. Indian J Basic Appl Med Res.2015;5:371–378.
21. Sujatha V, Sujatha C, Helena RajaKumari J, Sadana Revathi M. Evaluation of osteoporosis in postmenopausal women. Int J Recent Scientific Res. 2015; 6:5125–5127.
22. Al-Daghri NM, Aziz I, Yakout S, Aljohani NJ, Al-SalehY, Amer OE, et al. Inflammation as a contributing factor among postmenopausal Saudi women with osteoporosis. Medicine (Baltimore). 2017;96:e5780.
23. Mahdavi-Roshan M, Ebrahimi M, Ebrahimi A. Copper, magnesium, zinc and calcium status in osteopenic and osteoporotic post-menopausal women. Clinical Cases in Mineral and Bone Metabolism. 2015;12:18.
24. Mishra S, Manju M, Toora BD, Mohan S, Venkatesh BP. Comparison of bone mineral density and serum minerals in pre and post-menopausal women. Int J Clin Trials. 2015;2:85–90.
25. Castiglioni S, Cazzaniga A, Albisetti W, Maier JA. Magnesium and osteoporosis: current state of knowledge and future research directions. Nutrients. 2013;5:3022–3033.
26. Krumhar, Kim C., and Holly A. Johnson. "Composition for promoting healthy bone structure." U.S. Patent No. 6,447,809. 10 Sep. 2002.
27. Takeda E, Yamamoto H, Yamanaka-Okumura H, TaketaniY.Dietary phosphorus in bone health and quality of life. Nutrition Rev. 2012;70:311–321.
28. Calvo MS, Uribarri J. Public health impact of dietary phosphorus excess on bone and cardiovascular health in the general population. Am J Clin Nutr. 2013;98:6–15.
29. Al-Daghri NM,Yakout S, Al-Shehri E, Al-Fawaz H, Aljohani N, Al-Saleh Y. Inflammatory and bone turnover markers in relation to PTH and vitamin D status among Saudi postmenopausal women with and without osteoporosis. Int J Clin Exp Med. 2014;7:2812.
30. LEE, Jehoon; VASIKARAN, Samuel. Current recommendations for laboratory testing and use of bone turnover markers in management of osteoporosis. Annals of laboratory medicine, 2012, 32.2: 105-112.
31. Ilich JZ, Kerstetter JE. Nutrition in bone health revisited: a story beyond calcium. J Am Coll Nutr. 2000;19:715–737.
32. D'Amelio P, Cristofaro MA, Tamone C, Morra E, Di Bella S, Isaia G, et al. Role of iron metabolism and oxidative damage in postmenopausal bone loss. Bone 2008;43:1010–1015.
33. Huang X, Xu Y, Partridge NC. Dancing with sex hormones, could iron contribute to the gender difference in osteoporosis. Bone. 2013;55:458–460.
34. Chon SJ, Choi YR, Roh YH, Yun BH, Cho S, Choi YS, et al. Association between levels of serum ferritin and bone mineral density in Korean premenopausal and postmenopausal women: KNHANES 2008–2010. PloS One. 2014; 9:e114972.
35. Toxqui L, Vaquero MP. Chronic iron deficiency as an emerging risk factor for osteoporosis: a hypothesis. Nutrients. 2015;74:2324–2344.
36. Nawrot, T. S., Staessen, J. A., Roels, H. A., Munters, E., Cuypers, A., Richart, T, Vangronsveld, J."Cadmium exposure in the population: from health risks to strategies of prevention." Biometals 23.5 (2010):769-782.
37. Flaten, T. P., Aaseth, J., Andersen, O., & Kontoghiorghes, G. J. . "Iron mobilization using chelation and phlebotomy." Journal of Trace Elements in Medicine and Biology26.2-3 (2012): 127-130.
38. Weinberg ED. Role of iron in osteoporosis. Pediatr Endocrinol Rev. 2008;6: 81–85.
39. Weinberg ED. Iron loading: a risk factor for osteoporosis Biometals. 2006;19: 633–635.
40. Nakchbandi IA. Osteoporosis and fractures in liver disease: Relevance, pathogenesis and therapeutic implications. World J Gastroenterol. 2014;20:9427–9438.