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Investigation of Macrophage Erythroblast Attacher as New Protein and its Association with Vitamin D3 Levels among Hypertensive and Normotensive Osteoporosis Postmenopausal of Iraqi Women

Hussein Ali Al-Obaidi[1•] ; Fadhil J. Al-Tu'ma[1] ; Zena A. M. Al-Jawadi[2] ; Tamadhur S. M. Al-Hasnawy[3] and Saja Talib Ahmed[4]

[1]Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala/ Iraq. E. mail: f_altoma_56@yahoo.com

[2]Department of Chemistry, College of Science, University of Mosul, Mosul / Iraq. E-mail: zena_aljawadi@uomosul.edu.iq

[3]F.I.B.M.S, Rheumatology and Medical Rehabilitation, Al-Hassan Teaching Hospital, Kerbala Health Directorates / Kerbala – Iraq. E. mail: tamadhur.sabah@yahoo.com

[4]College of Pharmacy, Al-Zahraa University for Women/Iraq
Email:- saja.talib@alzahraa.edu.iq

[•]Corresponding Author:
Hussein Ali Al-Obaidi. Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala/ Iraq. Email: abitorab11@gmail.com

Abstract:

Background: Aged postmenopausal women have the greatest incidence rates of osteoporosis (OP), which is an increasing global health problem. The OP and fractures have a significant negative impact on a senior's quality of life and place a heavy financial and emotional strain on the patients and their family. The fundamental cause of all forms of OP is a lack of balance among bone formation and bone resorption. Osteopenia is a clinical term for a decline in Bone mineral density (BMD) that is below normal reference levels but not quite low enough to be diagnosed as osteoporotic. Hypertension (HTN) and OP are serious non-communicable public health issues due to the aging population and changing lifestyles. According to a number of studies, major risk factors for osteoporosis include age, gender, smoking, coronary heart disease, diabetes, essential hypertension, low estrogen levels, and others. Most minerals, including calcium (Ca) and magnesium (Mg), are necessary for good health.

The second most common intracellular cation is magnesium, which is the fourth most common cation in the body. Regarding its physiological function, magnesium is necessary for a variety of enzymatic processes. Magnesium is required for vitamin D3 activation. Serious organ dysfunctions can result from abnormally high or low amounts of either of these nutrients. Zinc, which functions as a local regulator of an osteoblast to establish the bony framework for organic matrix synthesis, is one of the minerals and trace elements found in abundance in bone.

Erythroblast attachment to macrophages is mediated by the membrane protein known as macrophage erythroblast attacher (MAEA). This includes granulocyte-macrophage colony-forming units, which are the monocytes of osteoclasts, and it plays a significant role in hematopoiesis in bone marrow.

Aims: The present work aimed to associate between a new MAEA protein, vitamin D3, and BMD in hypertensive and normotensive postmenopausal as control group.
Materials and Methods: We performed this case control study in department of chemistry and biochemistry, college of medicine by enrolling 80 subjects, (40) hypertensive postmenopausal women that have aged ranged between (56-86) years, and (40) normotensive postmenopausal women as control group, aged ranged between (50-76) years. All Patients had been in spontaneous menopause for at least, one year. In each subject we measured serum 25(OH)D3 level which determined by Cobas e 411 Analyzer System and serum total Mg, Ca, Zn and phosphorus which were analyzed by chemistry analyzer smart 120T/H, deionized water was used as a blank solution, Enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of serum MAEA. Body mass index (BMI) was calculated for each patient, and postmenopausal osteoporosis (PMOP) was validated using a dual energy x-ray absorptiometry scan (DEXA) in the lumbar spine regions (L1-L4 vertebrae).
Result: T-scor and BMD were significantly lower in hypertensive postmenopausal women when compared with control group (-3.11 ± 0.5 vs -1.61 ± 0.40), (0.70 ± 0.1 vs 0.86 ± 0.04 g/cm^2**),** P. value <0.05, while BMI was non-significant in hypertensive postmenopausal women when compared with control group (30.02 ± 4.23 vs 30.34 ± 4.64 kg/m^2) P. value>0.05. Magnesium and 25(OH)D3 were significant lower in hypertensive postmenopausal women compared with control group (2.21 ± 0.22 vs 2.31 ± 0.16 mg/dl), (26.67 ± 7.51 vs 31.03 ± 8.65 ng/ml), P. value<0.05. The MAEA protein was non-significant lower in hypertensive postmenopausal women when compared with control group (111.58 ± 29.06 vs 121.87 ± 23.15 pg/ml), P. value>0.05. Zinc was non-significant lower in hypertensive postmenopausal women when compared with control group (81.89 ± 13.15 vs 83.02 ± 7.43 µg/dl), P. value>0.05.
Conclusions: The study indicated that low magnesium is risk factors for osteoporosis in postmenopausal Iraqi women. Based on the results, hypertension assessment should be considered in postmenopausal women to prevent osteoporosis. This further supports the view that there is a biological link between the hypertension and osteoporosis. The 25(OH)D3 has positive correlated with the MAEA protein level. MAEA protein function in bone metabolism need to be studied in future research.
Keywords: MAEA protein, Postmenopausal Osteoporosis, Hypertension, BMD, Minerals, 25(OH)D3.

Introduction:

Aged postmenopausal women have the greatest incidence rates of osteoporosis (OP), which is an increasing global health problem. Osteopenia and poor bone microstructure are characteristics of the systemic bone disease osteoporosis, which raises the risk of fracture and bone fragility **(Coll *et al.*, 2021)**. The World Health Organization (WHO) defines it mainly in women as "the presence of a BMD less than or equal to - 2.5 standard deviations below the average bone mass of healthy 20-year-olds", which is carried performed using a specific radiological test, called bone mineral densitometry **(Elonheimo *et al.*, 2021).** Women have a roughly three times greater chance of

developing osteoporosis than do males **(Noh *et al.*, 2018).** Osteoporosis and fractures have a significant negative impact on a senior's quality of life and place a heavy financial and emotional strain on the patients and their family. The fundamental cause of all forms of osteoporosis is a lack of balance among bone formation and bone resorption **(Cai *et al.*, 2021).** The most prominent predictor for osteoporotic fractures is BMD, and the gold standard method for diagnosing OP is BMD based on dual-energy X-ray absorptiometry (DEXA). The therapy of osteoporosis/osteopenia and the evaluation of fracture risk both heavily rely on (DEXA)-based BMD **(Xu *et al.*, 2021).** The rapid drop in blood estradiol levels that occurs in women after menopause is directly linked to an increase in osteoclastic bone resorption. In postmenopausal women, low estrogen levels cause macrophages in the blood to generate osteoclastic cytokines, which activate Receptor activator of nuclear factor kappa B (RANK) and encourage osteoclast activation. Additionally, because estrogens no longer have their direct pro-apoptotic effects on osteoclasts, they live longer, which speeds up the loss of trabecular bone **(Golden, 2020)**. Osteopenia is a clinical term for a decline in BMD that is below normal reference levels but not quite low enough to be diagnosed as osteoporotic. OP is indicated by a T-score of -2.5 or below, whereas osteopenia is indicated by a result between -1 and -2.5. The OP is seen as a quantitative, rather than a qualitative, pathology of bone mineralization, and declining BMD values are indicative of an underlying disturbance in the microarchitecture of bone and osteopenia **(Karaguzel et al., 2010).**

Several studies indicate that age, gender, smoking, coronary heart disease, diabetes, essential hypertension, decreased estrogen level, and others are common risk factors for osteoporosis (**Pouresmaeili *et al.***, **2018**) . For older women, menopause is a physiological condition. Women can expect to live longer after menopause because to advancements in diagnosis and therapy. OP has been linked to aberrant Ca metabolism after menopause as well as a decline in BMD (**Kopiczko**, **2020**). The OP and HTN are two frequent disorders in older people due to an aging population and lifestyle changes, and both are significant challenges in clinical care and public health. The idea that the disorders have a similar etiopathology, involving low calcium intake and level, vitamin D and vitamin K deficiencies, and low or extremely high nitric oxide levels, was confirmed by prior epidemiological and biological research into the etiology of OP and HTN (**Yang *et al.***, **2014**). Whether there is a connection between hypertension and osteoporosis, however, is still up for debate. Numerous investigations have revealed a link between high blood pressure and low bone mineral density(**Ye *et al.***, **2017 and Yang *et al.***, **2014**). Although the precise processes are unknown, vitamin D is crucial for calcium homeostasis and bone remodeling (**Veldurthy *et al.***, **2016**). Low levels of 25(OH)D3 are harmful to bone, the overall population requires levels of 20 ng/ml which is well acknowledged. Reduced fracture risk was linked to this threshold (**Amrein *et al.***, **2020)**. Higher amounts (30 ng/ml), according to some research, are needed to preserve bone health. Hypocalcemia and secondary hyperparathyroidism are brought on by vitamin D insufficiency, which lowers BMD (**Priemel *et al.***, **2010**). Vitamin D3 insufficiency is a pandemic health issue that has been linked to a number of health issues and is well-documented in several regions of the world (**Holick *et al.***, **2008**). Vitamin D3 deficiency is a risk factor for fall

and fracture among post-menopausal women with OP (**Inderjeeth *et al.***, **2019**). Since only a few number of food sources are abundant in vitamin D, moderate sun exposure is the main source (**Suganthan *et al.***, **2020**). Most minerals, including Ca and Mg , are necessary for good health. They participate in the interaction of more than 300 enzyme activities and aid in the promotion of strong bones. Additionally essential for the development of strong bones and teeth, temperature control, nerve impulse transmission, and detoxification are these minerals (**Calderon-Garcia *et al.***, **2013**). Osteoporosis can result from magnesium depletion primarily through the following mechanisms:(1)altering the mechanism of hydroxyapatite affects bone mineralization, and enhancing bone turnover by stimulating the function of osteoclasts; (2) Inhibiting calcium homeostasis by affecting parathyroid hormone (PTH) and $1,25(OH)_2$-vitamin D, which may result in hypocalcemia; (3)promoting inflammation, by inflammatory cytokines stimulating remodeling and osteopenia and (4) promoting endothelial dysfunction (**Castiglioni *et al.***, **2013**). The skeleton's fundamental structural element is the calcium ion. The relevance of diet in preserving the health of bones and joints is being supported by increasing amounts of research. OP may be caused by a nutritional imbalance and endocrine problems **(Kumari *et al.*, 2018).**

The second most common intracellular cation and the fourth most prevalent cation in the body is magnesium (**Wolf *et al.***, **2003**). Mg has a vital physiological role in a variety of enzymatic activities (**Cowan**, **2002**). First, Mg can bind to an enzyme substrate to create a complex that the enzyme can interact with, such as the response of kinases with magnesium ATP (MgATP). Second, Mg directly binds to the enzyme, changing its structure and perhaps acting as a catalytic agent e.g., exonuclease, topoisomerase (**Zhang *et al.***, **2016**). The physiologic operations of many organs must be maintained with a sufficient level of magnesium to vitamin D3. To maintain healthy bone functioning, vitamin D3 helps to control the ratio of calcium to phosphate in the body (**Dusso**, **2014 and Razzaque**, **2009**). Magnesium is necessary for many different organs, including the skeletal muscles, heart, teeth, and bones. Additionally, magnesium is necessary for vitamin D3 activation. Serious organ dysfunctions may result from either of these minerals being consumed in excess (**Haq *et al.***, **2016**).

Minerals and trace elements like Zn are stored in bone (**Harkness *et al.***, **2019**). Zinc is found in the mineral that makes up bone, most likely in hydroxyapatite (**Chaudhry *et al.***, **2022**). ALP is one of the various osteogenic enzymes in which it participates. To create the bony framework for the creation of the organic matrix, zinc functions as a local regulator of an osteoblast. Zn stimulates osteoblast tyrosine kinase and enhances bone ALP activity. Osteopenia and bone development retardation are caused by a Zn deficiency in postmenopausal women (**Bhardwaj *et al.***, **2018**).
Numerous human cells, such as osteoblasts and osteoclasts, which are crucial for bone metabolism, express the gene macrophage erythroblast attacher (MAEA), which is found on chromosome 4p16.3. An important membrane protein called MAEA is encoded protein facilitates the adhesion of erythroblasts to macrophages (**Che *et al.***, **2019**). The MAEA has an significant role in hematopoiesis in bone marrow, including granulocyte-macrophage colony-forming units, which are the monocytes of osteoclasts (**Mao *et al.***, **2013**).

The present work aimed to investigate the level of a new MAEA protein which is the first study of Iraq regarding this protein and Vitamin D3, Mg, Ca, P, and BMD between hypertensive PMOP and control group to see if there is any influence caused an increase in fracture risk in osteoporosis patients.

Materials and Methods:

This case control study was performed in department of chemistry and biochemistry, college of medicine, university of Kerbala during Nov. 2021 to June 2022 by enrolling totally 80 subjects, (40) hypertensive postmenopausal women with age ranged between (56-86) years, and (40) normotensive postmenopausal women as control group with age ranged between (50-76) years. All Patients had been in spontaneous menopause for at least, one year. In each subject we measured serum vitamin D level which determined by Cobas e 411 Analyzer System and serum Total Mg, Ca, Zn and P, which were analyzed by chemistry analyzer smart 120T/H, deionized water was used as a blank solution, Serum MAEA levels were measured using Sandwich (ELISA) enzyme-linked immunosorbent assay kits (sunlongbiotech., China) according to the manufacturer's protocol. BMI was directly measured for each patient, BMD and T-scor by DEXA scan at lumbar spine regions (L1–L4 vertebrae) confirmed postmenopausal osteoporosis were included. hypertension patients were collected from Al-Hassan center for Endocrinology and Osteoporosis center at Al-Hussein Medical City, Kerbala Health Directorates/ Kerbala-Iraq. Inclusion criteria were as follows only female hypertensive and Normotensive postmenopausal as control group that have age of female ≥ 50 year were enrolled in the present study.

Individuals having a history of taking PMOP medication or medicine known to influence bone metabolism during the last six months were excluded from the study. Patients with conditions such severe malabsorption syndrome, chronic liver disease, inflammatory bowel disease, hypercalcemia, Paget's bone disease, active kidney stones, osteogenesis imperfect, and pituitary illness, all of which are known to influence bone metabolisms

Women were identified with surgical menopause, diabetic mellitus and hormone replacement therapy were excluded from the study. Patients with major underlying illnesses include rheumatoid arthritis, cancerous tumors, any form of thalassemia, and more. Patients with conditions such as lumbar spine fixation surgery history, ankylosing spondylitis and amputation surgery, and bone fracture were excluded from the study. Anthropometric data were recorded by interviews during health service provision. Diagnostically confirmed cases of PMOP attending the osteoporosis unit. Permission to conduct the study was obtained from the research ethics committee on human subject's research of Kerbala medical college, Iraq. After meeting the selection criteria, all of the instances were assessed and chosen at random. The osteoporosis unit received reports of the osteoporosis cases. They were asked to participate in the study and given information on the treatments utilized after being found to be suitable based on the selection criteria. There was informed consent received. The research included the patients who gave their permission. By interviewing the participants, further descriptive information about them, such as name, age, sex, and full background, was gathered. This information was then entered on a predesigned and pretested proforma.

BMD was determined by a diagnostic medical system Stratos Bone Densitometry equipment was designed and manufactured in France. The instrument operation and data interpretation were made according to manufacturer instructions. The interpretation was made according to BMD status, which was categorized as Osteoporotic (T-scor at or below -2.5), (osteopenic T-scor between -1 and -2.5), and normal (T-scor at above -1) postmenopausal women. Height and weight were measured at the time of DEXA measurement and BMI was calculated as the weight divided by the square of the height (kg/m^2). Five ml of blood sample was obtained by venipuncture from each patient during the morning (8-11 a.m.) and drawn into a gel tube, then allowed to stand at room temperature till the clot was formed. The blood tube was centrifuged, and serum was separated within 2 hours by centrifuging at 3,000 rpm for 10 min. All samples were stored in nonvacuum sterile tubes at −20 °C till further analysis.

The information was revealed in 2017 after IBM Corp.'s analysis. Version 25.0 of IBM SPSS Statistics for Windows. IBM Corp., Armonk, New York Unpaired Student's T-test was used to calculate the mean and standard deviation of the parameters for the two groups and compare them. Karl Pearson's correlation coefficient was used to calculate the link between the variables. A 5% level of significance (P 0.05) is used to determine statistical significance (**Di Leo *et al.*, 2020**).

Results:

Bone mineral density and T-scores were evaluated in 40 hypertensive postmenopausal and 40 normotensive postmenopausal women. Table (1) shows the Mean ± Standard Deviation (SD) of MAEA protein, age, BMI, T-scor, BMD, 25(OH)D3, Mg, Ca, Zn and P. Zinc was non-significant lower in hypertensive postmenopausal women when compared with control group (81.89 ± 13.15 vs 83.02 ± 7.43 µg/dl). The MAEA protein was non-significant lower in hypertensive postmenopausal women when compared with control group (111.58 ± 29.06 vs 121.87 ± 23.15 pg/ml), P. value<0.05.

Table 1: The Mean ± SD of the observed data determined in hypertensive and Normotensive postmenopausal women (control group).

Parameters	Reference Range	Hypertensive N = 40 Mean ± SD	Normotensive N = 40 Mean ± SD	P. value
Age, Year	-----	66.7 ±7.8	56.4 ±7.37	<0.01
BMI, kg/m^2	-----	30.02 ± 4.23	30.34 ± 4.64	0.74
T-scor	> -1	-3.11 ± 0.5	-1.61 ± 0.40	<0.01
BMD, g/cm^2	-----	0.70 ± 0.1	0.86 ± 0.04	<0.01
25(OH)D, ng/ml	30-100	26.67 ± 7.51	31.03 ± 8.65	0.02
Mg, mg/dl	1.7-2.5	2.21 ± 0.22	2.31 ± 0.16	0.04
Ca, mg/dl	8.6-10.3	9.29 ± 0.42	9.39 ± 0.44	>0.05
P ,mg/dl	2.5-4.5	3.91 ± 0.42	3.55 ± 0.65	<0.01
Zn, µg/dl	70-150	81.89 ±13.15	83.02 ± 7.43	>0.05
MAEA, Pg/ml	10-400	111.58 ± 29.06	121.87 ± 23.15	>0.05

Age and phosphorus were significantly higher in hypertensive postmenopausal women when compared with control group (66.7 ± 7.8 vs 56.4 ± 7.37 year), (3.91 ± 0.42 vs 3.55 ± 0.65 mg/dl), P. value<0.05. T-scor and BMD were significantly lower in hypertensive postmenopausal women when compared with control group(-3.11 ± 0.5 vs -1.61 ± 0.40),(0.70 ± 0.1 vs 0.86 ± 0.04 g/cm^2**),** P. value <0.05. The BMI was non-significant in hypertensive postmenopausal women when compared with control group (30.02 ± 4.23 vs 30.34 ± 4.64 kg/m^2), P. value>0.05. Magnesium and 25(OH)D3 were significantly lower in hypertensive postmenopausal women compared with control group (2.21 ± 0.22 vs 2.31 ± 0.16 mg/dl),(26.67 ± 7.51 vs 31.03 ± 8.65 ng/ml), P. value <0.05. Calcium was non-significant in hypertensive postmenopausal women compared with control group (9.29 ± 0.42 vs 9.39 ± 0.44 mg/dl), P>0.05.

Table 2: Relationship between parameters in Hypertensive PMOP

Correlation between parameters	Hypertensive Osteoporosis	
	r	Sig.
BMD and T.SCOR	0.997**	S
BMD and BMI	0.290	NS
BMD and Age	-0.224	NS
BMD and 25(OH)D3	0.321	NS
BMD and P	-0.373	NS
BMD and Zn	0.115	NS
T.SCOR and BMI	0.292	NS
T.SCOR and Age	-0.242	NS
T. SCOR and 25(OH)D3	0.315	NS
T.SCOR and P	-0.380	NS
BMI and Age	-0.367	NS
BMI and 25(OH)D3	-0.06	NS
BMI and Ca	-0.21	NS
BMI and Mg	-0.14	NS
BMI and P	0.436	NS
BMI and Zn	0.292	NS
Zn and BMD	0.115	NS
Zn and MAEA	-0.089	NS
Zn and Age	-0.449*	S
MAEA and 25(OH)D3	0.393*	NS
MAEA and BMD	0.157	NS
MAEA and T.SCOR	0.148	NS

*** SD: Standard Deviation; BMI: Body mass index; BMD: Bone mineral density, N: number, NS: non-significant; r: Pearson coefficient. ** Statistically significant(S) at p <0.01, *Statistically significant (S) at p <0.05.**

Table 3: Relationship between parameters in control group

Correlation between parameters	Control	
	r	Sig.
BMD and T.SCOR	0.577**	S
BMD and BMI	0.257	NS
BMD and Age	-0.194	NS
BMD and 25(OH)D3	0.598**	S
BMD and P	0.167	NS
BMD and Ca	0.221	NS
BMD and Mg	0.426	NS
BMD and Zn	0.048	NS
T.SCOR and BMI	0.520*	S
T.SCOR and Age	-0.394	NS
T. SCOR and 25(OH)D3	0.469*	S
T.SCOR and Mg	0.183	NS
T.SCOR and Ca	0.036	NS
T.SCOR and P	0.320	NS
T.SCOR and Zn	-0.182	NS
BMI and Age	-0.614**	S
BMI and 25(OH)D3	0.012	NS
BMI and MAEA	0.192	NS
25(OH)D3 and Age	-0.200	NS
25(OH)D3 and Ca	0.461*	S
25(OH)D3 and Mg	0.400	NS
Zn and MAEA	0.049	NS
MAEA and 25(OH)D3	0.511*	S
MAEA and BMD	0.423	NS

*Statistically significant(S) at p <0.05; NS : non-significant ;r :Pearson coefficient.** Statistically significant(S) at p <0.01

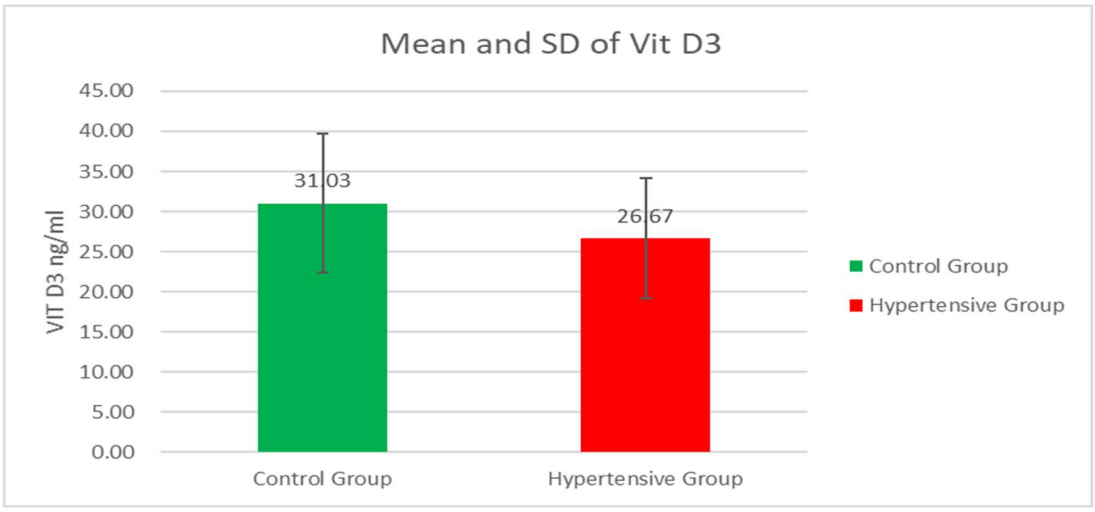

Fig 1: Compare between mean ± SD of 25(OH)D3 in hypertensive and control group.

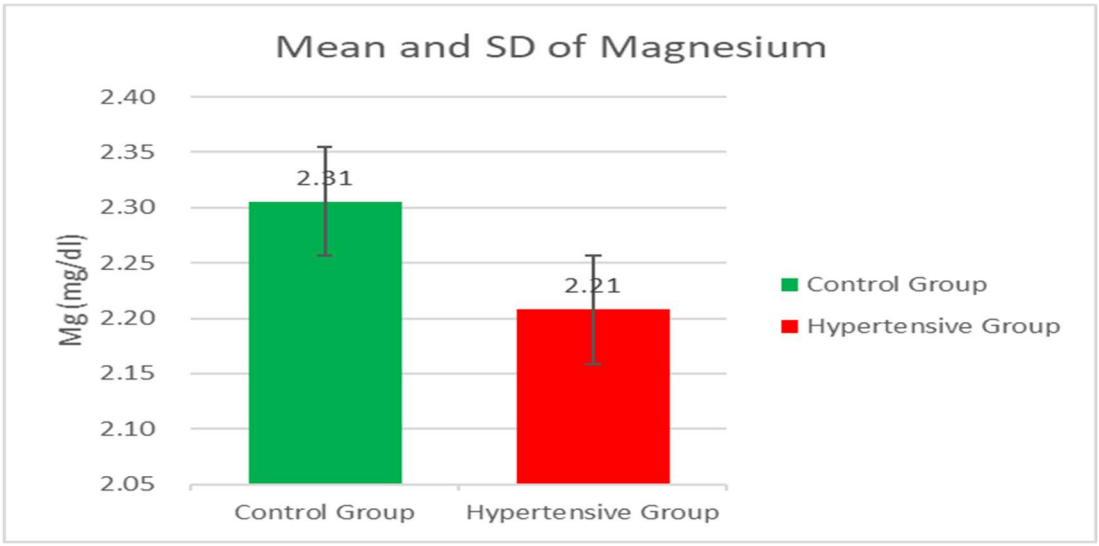

Fig 2: Compare between mean ± SD of magnesium in hypertensive and control group.

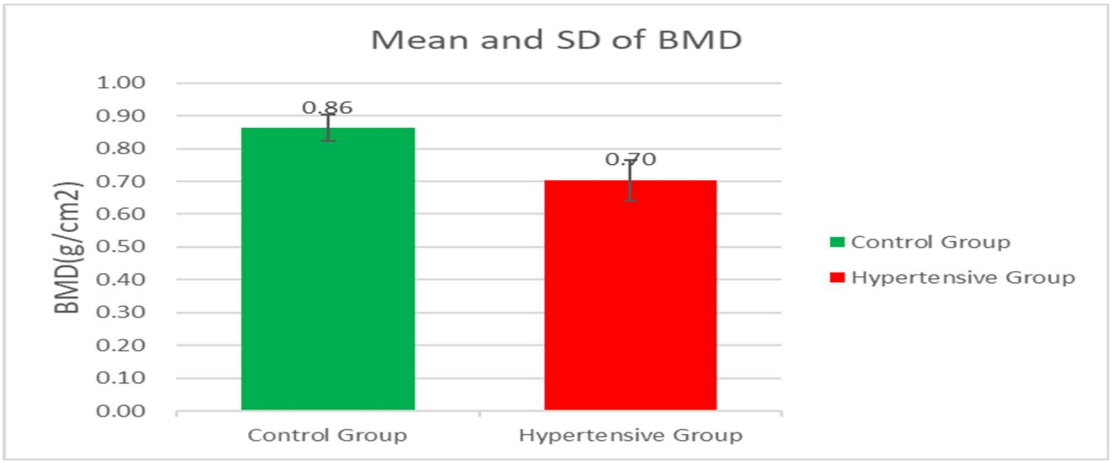

Fig 3: Compare between mean ± SD of BMD in hypertensive and control group.

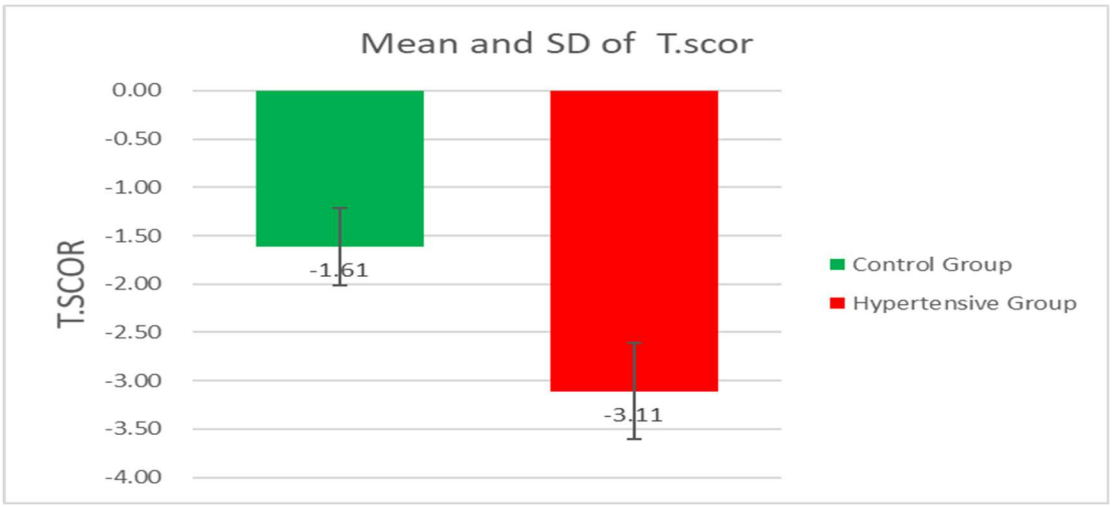

Fig 4: Compare between mean ± SD of T. SCOR in hypertensive and control group.

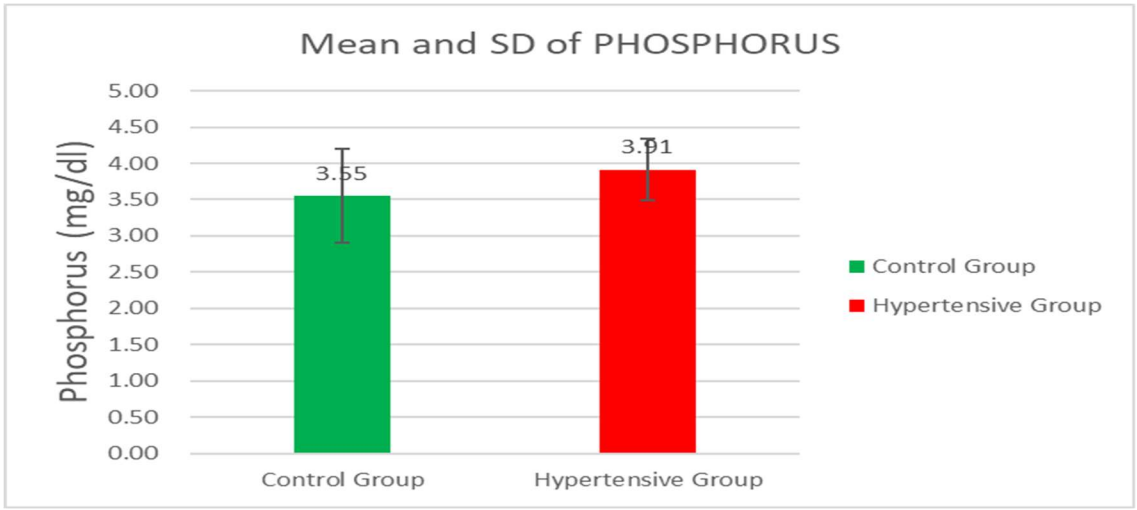

Fig 5: Compare between mean ± SD of phosphorus in hypertensive and control group.

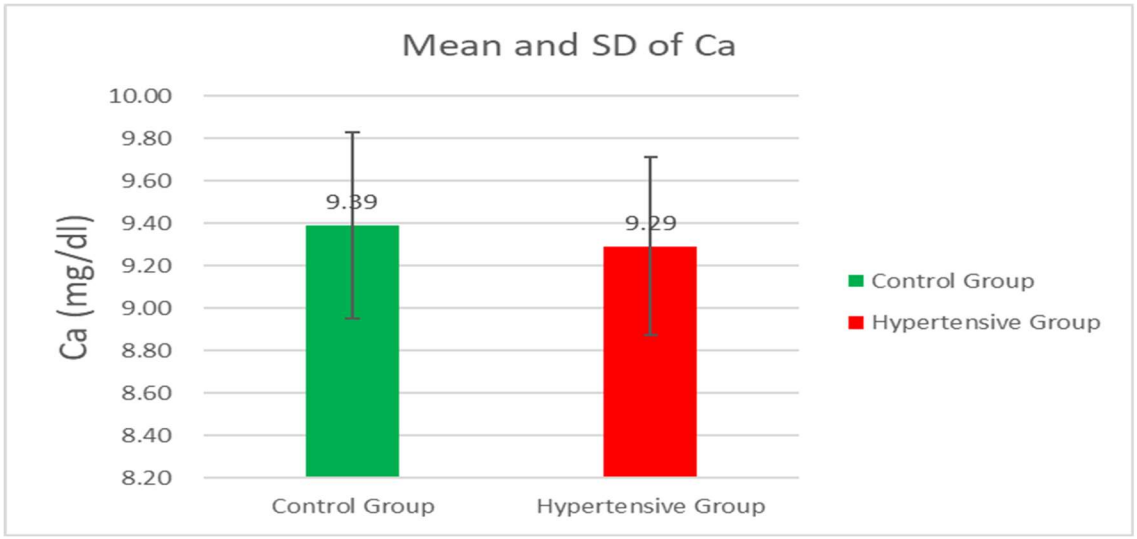

Fig 6: Compare between mean ± SD of calcium in hypertensive and control group.

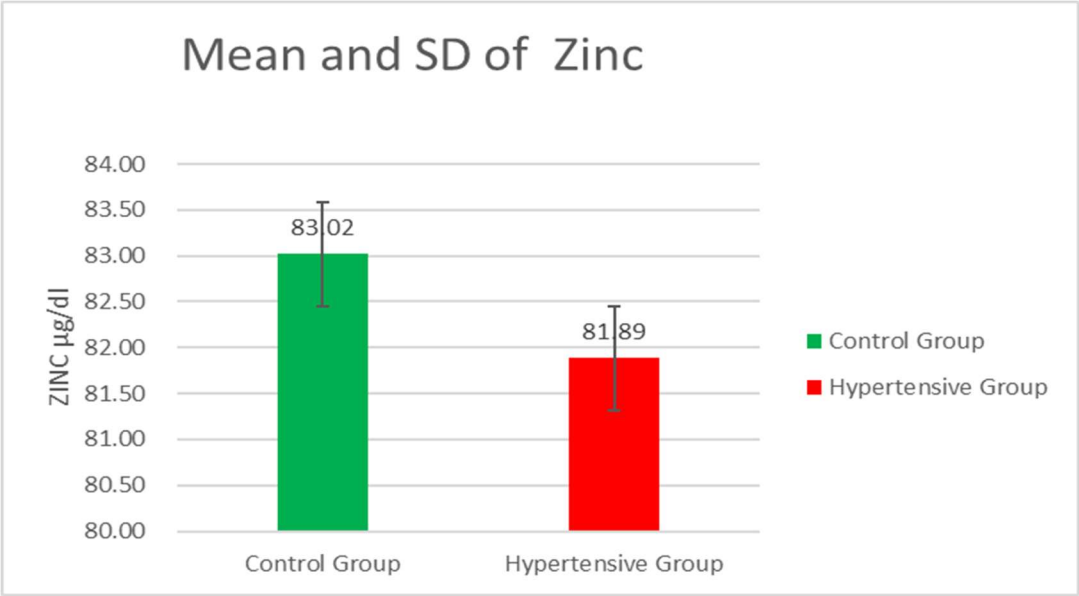

Fig 7: Compare between mean ± SD D of Zinc in hypertensive and control group.

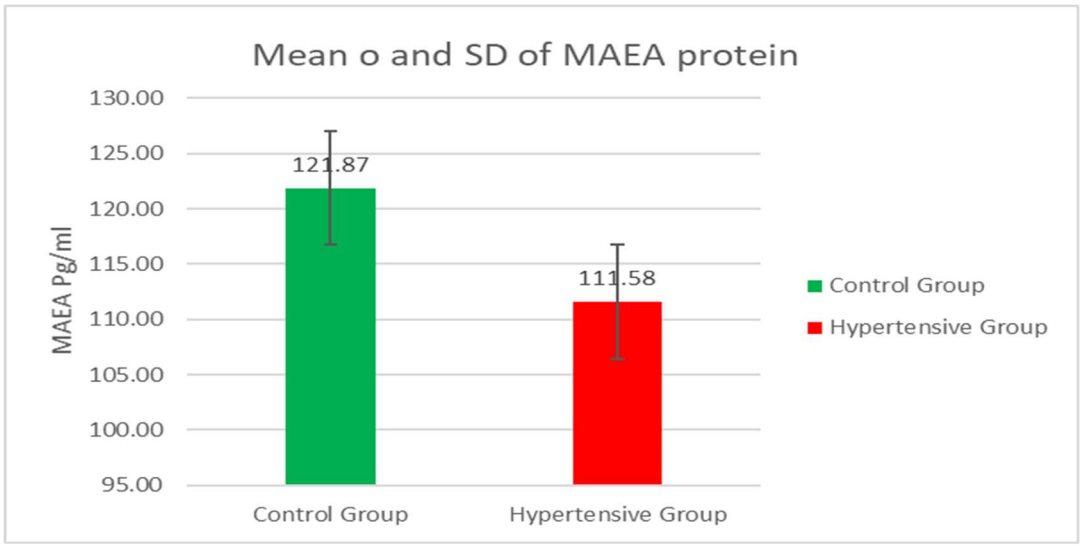

Fig 8: Compare between mean ± SD of MAEA protein level in hypertensive and control group.

Discussion:

The comparison of parameters characteristics indicated in table (1) show the mean of Age was higher statistical significant in hypertensive compared with normotensive (control) group ($P<0.01$). Age is considered as a risk factor for osteoporosis, elderly people are at higher risk for developing osteoporosis especially women that was agree with other investigation **(Cannarella *et al.*, 2019 and Xu *et al.*, 2020)** . Satoshi *et al*. found in his study that that age was significantly higher in osteoporosis patients and that female patients had a higher incidence of osteoporosis **(Tanaka *et al.*, 2018)**. As people age the rate of bone resorption by osteoclast cells exceeds the

rate of bone formation so bone weaken **(Owen *et al.*, 2018)**. This all happens due to: Inactive life style, Hormonal changes and Loss of calcium and other minerals in bone **(Blake *et al.*, 2021)**.

BMI indicated in table (1) show non-significant results differences between hypertensive and Control group(P>0.05),Both groups have a high BMI within the obesity category, but there is a statistically significant difference in BMD, and this may explain the effect of anabolic effect of adipokines secreted by adipose tissues **Kirk *et al.*, 2020)**. The results showed that subjects with high BMI have lower risk of developing osteoporosis than those with low BMI, these results agreement with some previous studies **(Hariri *et al.*, 2019)**. This can be explained by the favorable effects of greater mechanical loading on bone and increased estradiol levels due to enhanced conversion of androgen precursors to estrogen in the larger adipose tissue volume are thought to be the cause of the higher BMD in overweight/obese patients **(Xu *et al.*, 2020)**. When the BMI is stable, it becomes clear that there are other factors that affected the density of bone minerals, such as vitamin D, magnesium and zinc.

The results in table (1) BMD and T-scor were statistical significant lower in hypertensive group when compared with Control group(P<0.05). The result was in agreement with others **(Chai *et al.*, 2021)** and disagreement with others **(Javed *et al.*, 2012 and Lidfeldt *et al.*, 2002)**. Primary hypertension has an association with Ca metabolism, which causes loss of Ca and secondary activation of the parathyroid gland causing increased removal of Ca from bone **(Duque *et al.*, 2020 and Lidfeldt *et al.*, 2002)**. The presence of hypertension was found to be an independent predictor of low bone density. Long-lasting impairment effect of hypertension on calcium homeostasis may result in age-related excessive reduction of BMD and fracture **(Cappuccio *et al.*, 1999)**. The BMD is still the gold standard for the diagnosis of osteoporosis, but bone turnover markers can provide helpful information regarding the bone remodeling process **(Mederle *et al.*, 2018)**.

The results showed statistical significant lower difference in the mean serum Mg levels in hypertensive group when compared with control group (P. value<0.05) within normal range and consistent with Rekha *et al* which show a low level of magnesium in hypertension group compared to normotensive group but our study did not match them in terms of age, BMI, and sample size **(Rekha *et al.*, 2019)** and agreement with others **(Mederle *et al.*, 2018) ;** while our results were in disagreement with others **(Onor *et al.*, 2021)**. This result may be explain the impaired bone remodeling in osteoporosis, with reduced bone formation and increased bone resorption. The role of Mg in osteoporosis is also supported by studies, which demonstrated that Mg supplementation can increase bone density and stops bone loss in a significant proportion of subjects **(Castiglioni *et al.*, 2013)**.

The mean of the Ca in table (1) showed Calcium was no significant in hypertensive group when compared with control group (P>0.05). Serum calcium was normal in both hypertensive and control group because osteoporosis causes decrease in the total mineralized bone without a decrease in the ratio of bone mineral to the organic matrix. The results consistent with **(Yazici *et al.*, 2011)** and disagree with findings **(Sudjaroen *et al.*, 2022)** that found serum Ca significant decrease in osteoporotic compared to healthy groups**(Singh *et al.*, 2015)** who found serum Ca significant decrease in osteoporotic patients compared to non-osteoporotic groups.

In this study serum phosphorus showed significant increase in hypertensive group when compared with control group (P<0.05). The result consistent with work of **(Prabha *et al.*, 2015)** and inconsistent with **(Al-Hariri *et al.*, 2020 and Yazici *et al.*, 2011)**. Phosphorus and calcium are regulated mainly by two hormones PTH and active form of vitamin D3, there for any interference with action of PTH can lead to lowering serum calcium and increase of the serum phosphorus **(DiMeglio *et al*., 2019).**

The mean serum level of 25(OH)D3 was statistical significant lower in hypertensive group when compared with control group (P. value<0.05) and these result agreement with various reports **(Chai *et al.*, 2021)** and disagreement with others **(Marozik *et al.*, 2021 and Mederle *et al.*, 2018)**. Vitamin D3 effects have been widely investigated in various populations with regard to its possible effect on PMOP risk. The huge interest in vitamin D3 is explained primarily by its activity in calcium homeostasis, bone formation, and the regulation of bone mineral density **(Marozik *et al.*, 2021)**. In order to maintain healthy bone structure, stimulate bone mineralization, and control how osteoblasts and osteoclasts interact, vitamin D3 is primarily used in the body. Additionally, vitamin D3 can prevent angiogenesis, stimulate insulin synthesis, reduce renin production, induce terminal differentiation in cells, and limit cell proliferation **(Rosen, 2011)**. Our study showed no significant lower difference in the mean serum Zn levels in hypertensive postmenopausal women compared with control group (P. value>0.05) within normal range and consistent with different reports (**Suryana *et al.*, 2015 and Ferdous *et al.*, 2019**). The researchers indicated a low serum zinc level in hypertensive patients in both men and women (**Rahman *et al.*, 2021**), This finding was an agreement of previous studies (**Tiwari *et al.*, 2019 and Dueñas Ricaurte *et al.*, 2020**). In contrast some researchers indicated a high serum zinc level in hypertensive patients when compared with the control group (**Taneja *et al.*, 2007**). This disparity could be due to variation in sample size and study population.

The mean serum level of MAEA protein was statistical non-significant and lower in hypertensive postmenopausal women when compared with control group (P>0.05) and this result agreement with Cai *et al*. that who found MAEA protein level was significant lower in postmenopausal osteoporosis as compared with control group. No other study was found in the literature regarding MAEA protein in these diseases. The difference found with the other study may be due to the difference in the size of the sample, sample ethnic ancestry, as well as the different ages of women in the selected groups, and the difference in body mass index, in addition to other differences such as lifestyle and nutrition (**Cai *et al.*, 2021**). This recent study reported indicated the relationship between the MAEA gene with low bone mineral density in postmenopausal Japanese women (**Che *et al.*, 2019**) and this protein may be implicated in the emergence of osteoporosis in postmenopausal women and this need to more investigation in women after menopause in future. Our findings in distributions of MAEA protein blood levels indicated that the Vitamin D3 were significantly positive correlated with the MAEA protein level in control (P. value <0.05) and non-significant positive correlated in hypertensive postmenopausal women (P. value>0.05). The statistical analysis of each independent parameter indicated that there

were no significant correlations between the BMI and BMD in control and hypertensive groups (p. value >0.05) and (p. value >0.05), respectively.

This result consistent with **(Matijević *et al.*, 2016 and Al-Taie *et al.*, 2014)** and inconsistent with other **(Raba'a, 2013)** who found significant positive correlation between BMD and BMI. The analysis of MAEA protein indicated no significant positive correlations with BMD in control and hypertensive groups (P. value>0.05). The study also observed positive significant correlation between vitamin D and BMD in control group (p.value <0.05) and this agreed with **(Singh *et al.*, 2015 and Liu *et al.*, 2019)** and also non-significant correlation between BMD and vitamin D3 in hypertensive group but the same direction (p. value >0.05) and disagreement with others (**Abdelgadir *et al.*, 2016)**. The study also observed strong significant positive correlation between T-scor and Vitamin D3 in control group (p. value <0.05) and this agreement with certain report **(Lowe *et al.*, 2011)** and disagreement with others **(Hariri *et al.*, 2019).** In hypertensive group non-significant correlation between T-scor and vitamin D3 but the same direction may be because study's population, sample size, and demographic characteristics differ from other similar studies. The study also observed non-significant negative correlation between BMD and age (P. value>0.05) and this result agreement with **(Makker *et al.*, 2008)**. Lee *et al* found inversely correlated between BMD and age **(Lee *et al.*, 2020)**. Our study observed significant positive correlation between BMI and T.scor (p. value<0.05) in control group and this agreement with various articles **(Hariri *et al.*, 2019** and **Doğan *et al.*, 2010)** and non-significant in hypertensive group. The Mg levels was non-significant positive correlated with BMD in control group values (P>0.05) same direction with Mederle *et al* who found Positive correlation between BMD and magnesium **(Mederle *et al.*, 2018)** and disagreement with **(Farsinejad-Marj *et al.*, 2016)**, whereas, Ca levels was non-significant positive correlated with BMD in two groups and this result disagreement with **(Liu *et al.*, 2019)**, and agreement with **(Ali, 2018)** who found non-significant correlation between Ca and BMD but opposite direction. Phosphorus and magnesium are among minerals that have been proposed as having an important role in bone metabolisms. Phosphorus, as phosphates combine with calcium ions to form hydroxyapatite, the major inorganic molecule in teeth and bones.

Conclusions:

Hypertensive postmenopausal women had inadequate vitamin D3 levels and high BMI have prone to osteoporosis. Based on the results, hypertension assessment should be considered in postmenopausal women to prevent osteoporosis. This further supports the view that there is a biological link between the hypertension and osteoporosis. The age, low vitamin D3 and obesity are risk factors for osteoporosis in postmenopausal Iraqi women. The hypertensive group has BMD lower than in normotensive group. The mean of Mg, Zn and Ca levels more in normotensive group than hypertensive group may contribute to high BMD and may be a protective factor against osteoporosis and the positive correlation between BMD and Ca, Mg, Zn and also positive correlation between BMD and vitamin D3 confirm it. Vitamin D3 has positive correlated with the MAEA protein level. MAEA protein function in bone metabolism need to be studied in future

research. In the future, should be conducted to thoroughly investigate the genetic architecture of MAEA and its effects on postmenopausal Iraqi women.

Limitations:

The current study focused on a particular group and was hospital-based. As a result, the findings cannot be applied generally. It was not investigated how lifestyle and environmental variables may affect bone mineral density.

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