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2 .parathyroid hormone (PTH)

The value of mean ± S.D (73.31 ± 39.11) in ($p \leq 0.001$) in patients with osteoporosis while the value of mean ± S.D (30.01 ± 18.85) in control group it mean that there was a very high significant difference in the value of p where ($p \leq 0.001$) in patient with osteoporosis comparing with control because the parathyroid hormone (PTH) acts to regulate calcium in the bloodstream ,the parathyroid hormone PTH promotes the release of calcium from the large reservoir in the bones.[8] [20]

That the presence of sufficient amounts of vitamin D3 maintain the level of calcium in the blood, and prevents the high level of hormone thyroid gland which stimulates the release of calcium from the bones and lead to bone resorption. [21] [22] [23]

Bone resorption is a process in which bones are naturally destroyed by osteoclasts, which are indirectly stimulated by PTH, as the parathyroid hormone stimulates bone resorption indirectly because osteoclasts do not have a PTH receptor. Instead, PTH is related to osteoblasts, which are the cells responsible for bone synthesis. The association between pth and osteoblasts stimulates the formation RANKL (Receptor activator of nuclear factor kappa-B ligand),The secretion of Osteoprotegerin (OPG) inhibits RANKL by binding with it where free OPG is associated with RANKL competitively. This binding prevents RANKL from interfering with RANK (Receptor activator of nuclear factor κ B) . The binding between RANKL and RANK (facilitated by the decreased amount of OPG available for excess RANKL binding) ,The binding of RANKL to RANK stimulates the osteoclast and leading to bone resorption . [24]

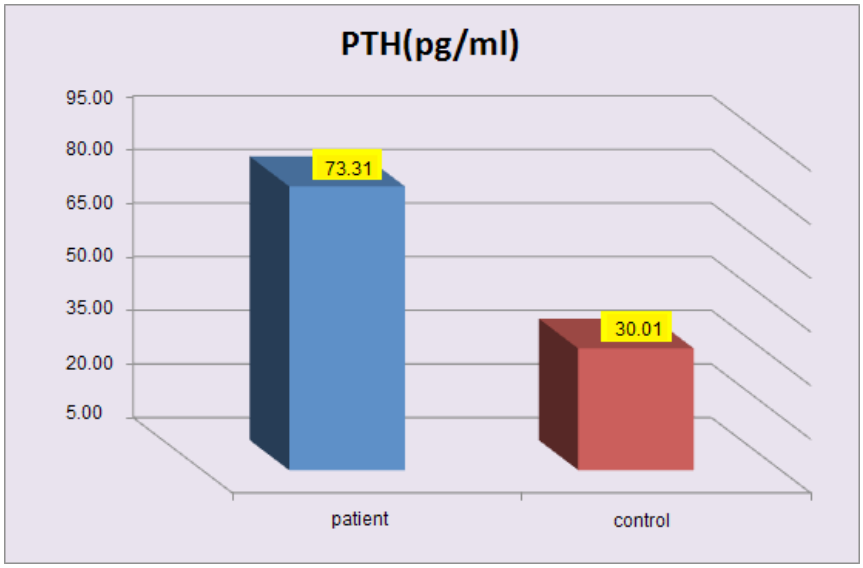


Figure (5)

Parathyroid hormone in the serum group of women with osteoporosis and control group

3. Malabsorption: Vitamin D3 deficiency occurs in people with gastrointestinal disorders.(14) (15)
4. Cholecystectomy leads to a malfunction in the absorption of vitamin D3 where the gallbladder get up stores and concentrates the yellow substance produced by the liver and has a significant role in the absorption of fat-soluble vitamins, including vitamin D3.(16) (17)

Vitamin D3 maintains the level of calcium and phosphorus in the bloodstream as it stimulates absorption of calcium in the intestinal wall by stimulating the formation of calcium-binding protein in the intestinal wall, Vitamin D works on the transfer of mineral elements from the bloodstream to the bone, and mineral elements have a significant role in bone mineralization, and lack of vitamin D leads to the lack of mineral elements in the bone, and lack of mineral elements is associated directly with the mineral density of bone, as the lack of mineral elements leads to reduce the density Mineral bone density ,and bone mineral density decrease lead to reduced bone strength leading to osteoporosis.(18) (19)

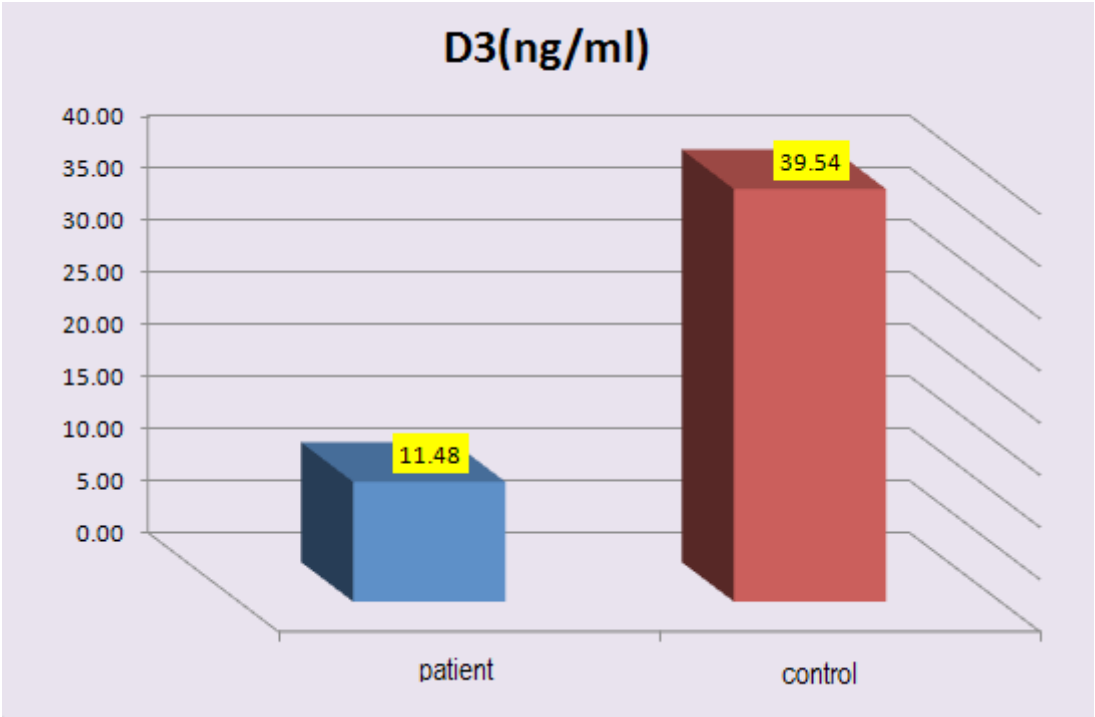


Figure (4) vitamin D3 in the serum group of women with osteoporosis and control group

Result and Discussion:

A statistical analysis was performed to compare results of the control group and women with osteoporosis , the following results were obtained.

Table (1) Levels of Vit.D3 and Parathyroid hormone in patients with with osteoporosis comparing control group

Factor		Mean	.Std Deviation	T Test	P_value
Vit. D_3 (ng/ml)	patient	11.48	2.73	-19.368	0.000
	control	39.54	8.63		
PTH (pg/ml)	patient	73.31	39.11	4.115	0.000
	control	30.01	18.85		

1. Vitamin D_3

The value of mean ± S.D (11.48 ± 2.73) in ($p \leq 0.001$) in patients with osteoporosis while the value of mean ± S.D (39.54 ± 8.63) in control group it mean that there was a very high significant difference in the value of p where ($p \leq 0.001$) in patient with osteoporosis comparing with control where Low levels of vitamin D_3 have been observed in patients with osteoporosis. There are several reasons for low levels of vitamin D_3 in the body. The most important reasons were observed in the cases Which has been dealt with:

1. Malnutrition: Vitamin D deficiency occurs if the diet is deficient in amounts of meat, fish and eggs, and low consumption of cereals, where vegetarians are found to have vitamin D deficiencies. reason for the nature of the diet followed. (10) (11) (12)
2. Low exposure to the sun: - Wear heavy clothing has a major role in reducing the amount of skin exposed to UV rays and reduce the production of vitamin D3. Clothing covering a large part of the skin, associated with low levels of vitamin D3 and increased prevalence of vitamin D3 deficiency.(12) (13)

Reagent Preparation:

Wash Buffer: Were dilute contents of wash solution concentrate by adding the contents of the bottle (25 ml , 20x) to 475ml of distilled or deionized water.

Procedure:

1. Format the microplates wells for each serum reference, control and patient specimen to the assayed in duplicate.
2. pipette 0.025 ml (25µl) of the appropriate serum reference Calibrator, control and specimen into assigned well.
3. Were added 0.050ml (50µl) of the biotin reagent to each well.
4. Were add 0.050ml (50µl) of the conjugate reagent to each well.
5. Incubated 90 min at room temperature on the a plate shaker (500- 600 rpm).
6. Discard the contents of the microplate by decantation or aspiration.
7. Were add 0.300ml (300µl) of wash buffer, repeat three (3) additional times for a total of four (4) washes
8. Were add 0.100 ml (100µl) of TMB substrate to all wells.
9. Incubated at room temperature for 15 min.
10. Were add 0.050 ml (50µl) of stop solution to each wells.
11. read the absorbance in each well at 450 nm in a microplate reader.

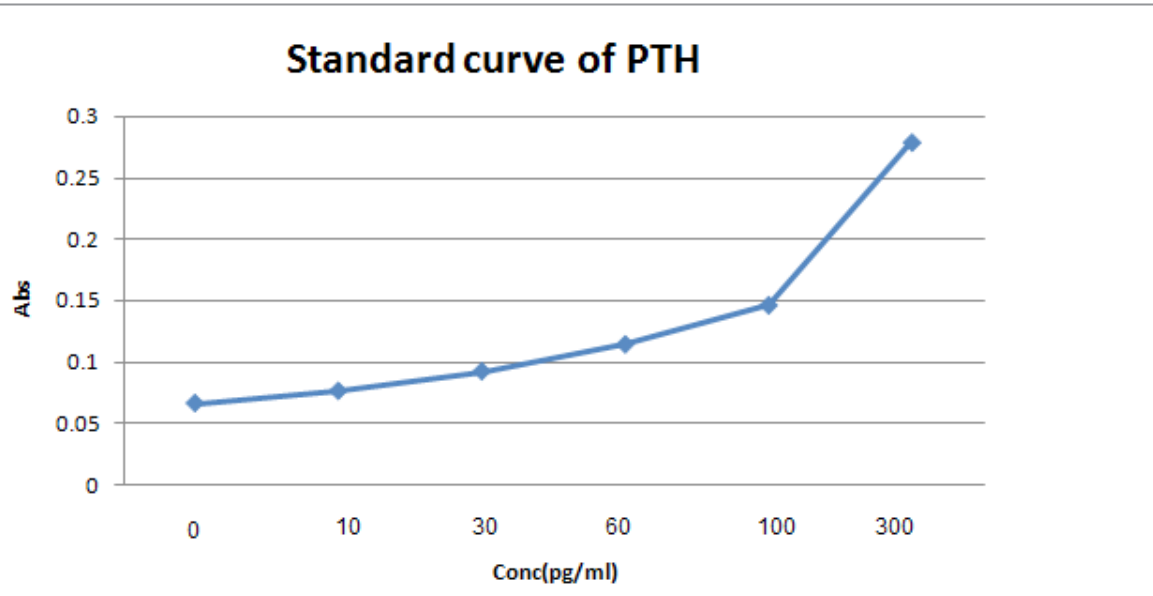


Figure (3) The standard curve for Parathyroid hormone estimation

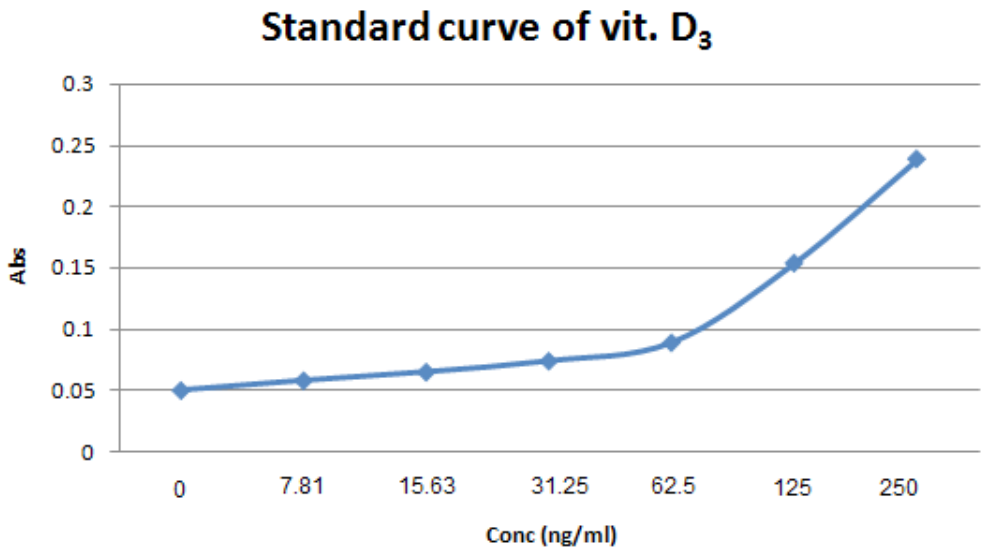


Figure (2) The standard curve for vitamin D3 estimation

2. parathyroid hormone (PTH) parathyroid hormone (PTH) Monobind Reagent: Kits for determination of , USA

PTH Calibrators	0.5 ml/vial	Six vials of references for PTH at levels 0(1),10(2),30(3),100(4),300(5)
PTH Controls	0.5 ml/vial	Two vials of references controls for PTH
Anti-PTH Biotin Reagent	7 ml/vial	One vial containing Anti-PTH biotin reagent
PTH Enzyme Conjugate	7 ml/vial	One vial containing Anti-PTH Conjugate reagent
Streptavidin Coated Concentration	96 wells	One 96-wells microplate coated with Streptavidin
Wash Solution Concentration	25 ml/vial	See reagent preparation section
TMB Substrate	12 ml/vial	One vial containing tetramethylbenzidine (TMB)
Stop Solution	12 ml/vial	One vial containing a strong acid
Product Instruction		

of the 500 ng/mL stock solution to the first tube and were mixed up to produce a 250 ng/mL working solution. Pipette 500uL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.

4. Biotinylated Detection Ab working solution: Were Calculated therequired amount before the experiment (50 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use,were diluted the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent.
5. Concentrated HRP Conjugate working solution: Were Calculated the required amount before the experiment (100µL / well). In preparation, slightly more than calculated should be prepared .Were diluted the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

Procedure:

1. Were added the Standard working solutionto the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (50 uL for each well). Were added the samples to the other wells (50 uL for each well). Immediately were added 50µL of Biotinylated Detection Ab working solutionto each well. Covered by the plate with the sealer provided in the kit. Incubated for 45 min at 37Cº.
2. Aspirated or decanted the solution from each well ,were added 350 uL of wash bufferto each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeated this wash step 3 times.
3. Were added 100 µL of HRP Conjugate working solutionto each well. Covered by with the Plate sealer. Incubated for 30 min at 37Cº.
4. Aspirated or decanted the solution from each well, repeated the wash process for five times as conducted in step 2.
5. Were added 90 µL of Substrate Reagentto each well. Covered by a new plate sealer. Incubated for about 15 min at 37°C.
6. Were added 50 µL of Stop Solutionto each well.
7. Were determined the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Methods :

1.Vitamin D3

Reagent: Kits for determination of Vitamin (D3) ELABSCIENCE , USA.

Micro ELISA Plate (Dismountable)	8 wells ×12 strips
Reference Standard	2 vials
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 μL
Concentrated HRP Conjugate (100×)	1 vial, 120 μL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	1 vial, 30 mL
Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Product Instruction	

Reagent Preparation:

1. Were bring all reagents to room temperature (18~25^{O}C) before use
2. Wash Buffer: Were dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepared 750mL of Wash Buffer.
3. Standard working solution:- Were added 1.0 mL of Reference Standar and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolved fully, mixed it thoroughly with a pipette. This reconstitution produces a working solution of 500 ng/mL. Then were maked serial dilutions as needed .The recommended dilution gradient is as follows: 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 0 ng/mL.

Take 7 EP tubes ,were added 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL

body can make withdrawals as needed to maintain the calcium intake in the blood at appropriate levels Parathyroid hormone is the key to remove calcium from the bone [8].

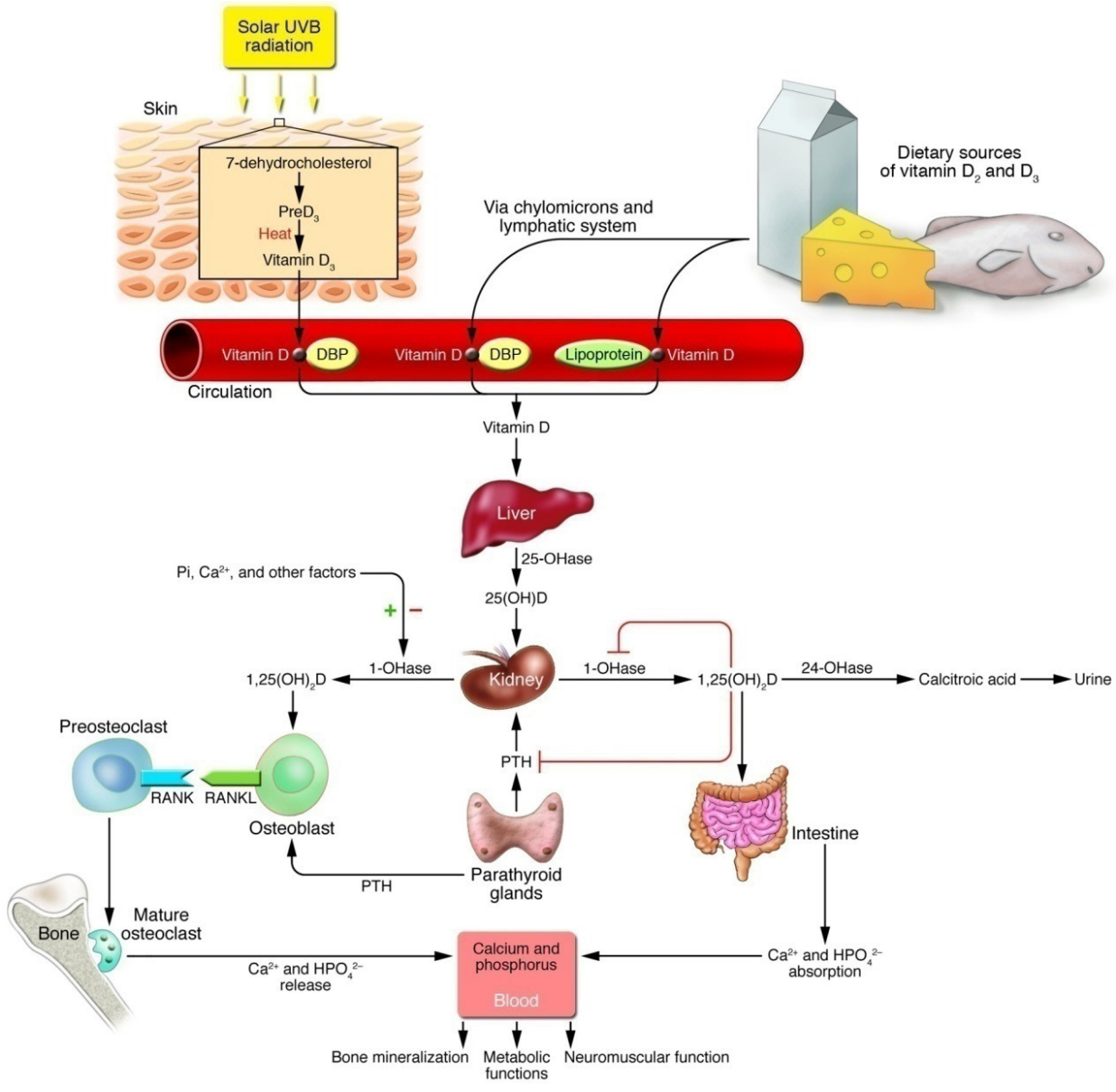


Figure (1) explain the role of vitamin D and parathyroid hormone in regulating blood calcium in the human body [9]

Introduction:

Osteoporosis is one of the most important bone diseases ,characterized by decreased bone density and microarchitectural deterioration of bone tissue ,When bones become fragile and thin, they are prone to fracture[1][2].

Osteoporosis has been labeled a "silent disease" and a more amenable to prevention than treatment. It is silent, because one is unaware of bone loss process until it becomes clinically evident as a fracture[3][4].

1. Vitamin D_3

Vitamin D is a fat - soluble vitamin, sometimes called sun Vitamin, because its composition includes the exposure of sterols compounds to ultraviolet light. there are two important type of Vitamin D , Vitamin D_2 or calciferol and vitamin D_3 or cholcalciferol.[5]

Vitamin D_2 can be obtained when the provitamin D_2 called ergosterol is exposed to sunlight or ultraviolet radiation. Vitamin D_3 can also be obtained when the provitamin D3 called 7-Dehydrocholesterol is exposed to sunlight or ultraviolet radiation.[6]

In the liver, cholcalciferol is converted into 25 - hydroxy cholcalciferol (25HCC) and the latter compound is converted in the kidney into 1,25- dihydroxy cholcalciferol (1,25DCC), called calcitriol, which is the effective form of vitamin D_3.[7]

This vitamin has a direct effect on the calcification of bones and teeth. It stimulates the absorption of calcium in the intestine ,It stimulates the production of a transport protein called calcium binding protein . It also stimulates the Renal absorption of calcium and phosphate.

2.parathyroid hormone (PTH)

Parathyroid hormone is a hormone secreted by the parathyroid glands which is important in bone reshaping, a continuous process in which bone tissue is reabsorbed and reconstructed over time. Parathyroid hormone is secreted in response to low blood calcium levels (Ca^{+2}). Parathyroid hormone indirectly stimulates osteoclast activity within the bone marrow, trying to release more calcium ion (Ca^{+2}) into the blood to raise blood calcium levels, Parathyroid hormone regulates calcium in the blood through its effect on bones, kidneys and intestines , Bones act as a calcium bank, from which the

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The influence of Vitamin D3 and Parathyroid hormone in women with Osteoporosis in Al-Anbar Governorate

Ehsan H. Rasheed, Wajeeh Y. Mohammed Alani and Ismail K. Farhan.
University of Anbar ,College of Science ,2019

Abstract:

In this study were studied one of the most important bone diseases is osteoporosis, Osteoporosis affects half of the world's women and leads to fractures ,the aim of the study to know the effect of vitamin D and parathyroid hormone on osteoporosis . This study was conducted on women in Anbar Governorate. The study includes (60) women aged 25-65 years. 15 women were taken as control groups aged 25- 35 years and 45 women with osteoporosis.

The results of our study showed a decrease in vitamin D3 concentration of serum in women with osteoporosis, the results also showed an increase in parathyroid hormone concentration of serum in women with osteoporosis.