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Study the Relationship between Ca Ions Level and Osteoporosis in Thyroid Diseases Patients

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Abstract:
D3 is a steroid (fat soluble) vitamin, produced in the skin. D3 receptors found in most tissues in the body and has many roles especially in the regulation of calcium and phosphorous homeostasis. Recently, many research suggest that deficiency in vitamin D3 has a role in many diseases with autoimmune disorders, many cancers, heart diseases and death. Lowering in vitamin D3 levels also associated with autoimmune thyroid disease (AITD). Our study aimed to find the correlation between deficiency in vitamin D3 and osteoporosis, study a relationship of D3 deficiency and thyroid disease and to find the effect of thyroid disease on osteoporosis. The experimental groups used consist of 70 patients age ranged from (18-84) years old, they are divided into three groups: group (1): (group with normal D3), group (2): patients with D3 deficiency without osteoporosis and group (3): patients with D3 deficiency and osteoporosis. Serum Vit.D3,Ca,T3,T4 and TSH were measured and osteoporosis was diagnosed by bone densitometry method.Our results showed that the Mean ± SE values of T4,Ca and D3 were lower in patients suffering from D3 deficiency with osteoporosis than that of control group and patients with D3 deficiency without osteoporosis ($P \leq 0.01$), a significant positive correlation coefficient of T4 with Ca and vit.D3 ($P \leq 0.01$), but a no significant positive correlation coefficient of T3 with TSH and vit. D3, no significant negative correlation coefficient of T3 with Ca. We concluded that Iraqi patients with hypothyroidism suffering from deficiency in vitamin D3 and Ca that in turn leads to osteoporosis. Vitamin D and Ca levels screening was recommended to all Iraqi patients with hypothyroidism to avoid osteoporosis if the deficiency continued.
Keywords: Osteoporosis, Osteomalacia, hashimotos thyroiditis, graves disease

INTRODUCTION

Vitamin D3 is a type of vitamins that are soluble in fats and plays an important rolre in increasing absorption of calcium by intestine, also absorption of magnesium and phosphate.
The main source of this vitamin is the synthesis in the deeper layers of skin by a chemical reaction in presence of sun light (specifically the ultra violet radiation). We can obtaining vitamin D3 from the diet or from supplements such as the fatty fish flesh that contain a large amounts of this vitamin (Watkins *et al*.,2015). Vitamin D_2 differs from vitamin D_3 in the chemical structure especially in the side chain of vitamin D_2 that contains a double bond that binds carbons 22 and 23, D_2 also contains a group of methyl on the carbon 24. Vitamin D also affects immune system, and vitamin-D receptors (VDRs) are expressed in several white

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blood cells, including monocytes and activated T and B cells. A food that contains insufficient amounts of vitamin D combined with inadequate exposure to sunlight causes a deficiency in this vitamin. Children that suffering from severe deficiency in vitamin D have a risk of rickets, a disease includes bones softening and weakening, which is considered rare disease in developing world (Norman, 2008). The deficiency in D3 vitamin is found worldwide in the old people and common in childhood and also in adults (Munns, 2016). Vitamin D deficiency in pregnant women may cause either bone disease before birth or impairment in the quality of bones after birth (Elidrissy, 2016).

The most important disease in adults that results from the deficiency in vitamin D is osteoporosis. Characteristics of osteoporosis are bones softening and weakening which in turn leading to fragility of bones which if continued and untreated may cause bone fractures. Another disease that infects bones is osteomalacia that results from reduces absorption of calcium and increases loss of calcium from bones. Chronic musculoskeletal pain may results from osteomalacia (Gaikwad, 2016).

Aims of the study:

1- Study the relationship between vitamin D3 deficiency and osteoporosis.
2- Study the relationship between D3 deficiency and thyroid disease.
3- Find the effect of thyroid disease on osteoporosis.

MATERIALS AND METHODS

The experimental groups: The experimental groups used consist of 70 patients age ranged from (18-84) years old, they are divided into three groups: group (1): consist of 20 sample of control group (group with normal D3) , group (2) consist of 30 sample of patients with D3 deficiency without osteoporosis and group (3) consist of 2o sample of patients with D3 deficiency and osteoporosis.

Blood samples collection: Five millileter (ml) of blood samples were aspirated from each patient and control cubitus vein to test the level of D3, T3,T4,TSH and calcium.

Laboratory investigations:

2.3.1. Measuring of T3, T4 and TSH hormones in the serum by using **TOSOH AIA 360** full automated (Japan), depending on *Florescent enzyme immunoassay technology* (FEIAT).

2.3.2. Measuring of D3 vitamin and Ca in the serum:

The test performed by using **Roche Cobas e411** a product of Roche Company (Us FDA approved).

Diagnosis of osteoporosis:

Osteoporosis was diagnosed by dual-energy x-ray absorptiometry (DEXA).

Statistical analysis:

SAS or the Statistical Analysis System (2012) was used in the statistical analysis. Least significant difference –LSD test (Analysis of Variation-ANOVA) was also used to find the significant comparision between means. The correlation coefficient between variables was also used in our study.

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RESULTS

Our results in table (1) explained that the Mean ± SE values of T4 were lower in the patients suffering from D3 deficiency and osteoporosis than that of control group and patients with D3 deficiency without osteoporosis (P≤0.01) with LSD value of (1.297). The table also showed a non significant difference among the three groups in the Mean ± SE of T3 with LSD value of (0.184), we also observed another no significant difference among three groups in the Mean ± SE of TSH with LSD value of (0.469).

Table. 1: Comparision between different groups in the level of hormones

Groups	Mean ± SE		
	T4(nanogram /milliliter)	T3(nanogram /milliliter)	TSH(nanogram /milliliter)
Control group: Normal D3 level	11.16 ± 0.24 a	1.350 ± 0.07 a	2.562 ± 0.18 a
Patients with D3 deficiency without osteoporosis	11.33 ± 0.34 a	1.393 ± 0.05 a	2.567 ± 0.12 a
Patients with D3 deficiency and osteoporosis	7.95 ± 0.71 b	1.330 ± 0.07 a	2.421 ± 0.20 a
LSD value	1.297 **	0.184 NS	0.469 NS
P-value	10^{-4}	0.760	0.789
Means that having letters (a and b) in same column were significantly different. ** ($P \leq 10^{-2}$)			

Our results in table (2) demonstrated that the Mean ± SE of Ca (6.43 ± 0.23) in patints with D3 deficiency and osteoporosis was significantly lower than the Mean ± SE of Ca in control group (8.63 ± 0.17), and patients with D3 deficiency without osteoporosis (8.26 ± 0.16) at (P≤0.01). The LSD value was (0.540), but the Mean ± SE of vitamin D3 was significantly lower in patients with D3 deficiency and osteoporosis (7.04 ± 0.27) than that of patients with D3 deficiency without osteoporosis and control group (P≤0.01), table (2) also showed that Mean ± SE of vitamin D3 in patients with D3 deficiency without osteoporosis (12.58 ± 0.52) was significantly lower than that of control group (23.18 ± 0.54) at (P≤0.01).

Table 2: Comparison between difference groups in Ca and Vit. D3

Groups	Mean ± SE	
	Ca(mg/dl)	Vit.D3(ng/ml)
Control group: Normal D3 level	8.63 ± 0.17 a	23.18 ± 0.54 a
Patients with D3 deficiency without osteoporosis	8.26 ± 0.16 a	12.58 ± 0.52 b
Patients with D3 deficiency and Osteoporosis	6.43 ± 0.23 b	7.04 ± 0.27 c
LSD value	0.540 **	1.408 **

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P-value	0.0001	0.0001
Means that having letters (a,b and c) in same column were significantly different**.** ** ($P \leq 10^{-2}$).		

Table (3) showed that there were no significant differences between males and females in the level of Mean ± SE of T4, T3, TSH and Ca, but the Mean ± SE of D3 was significantly lowered in females (9.97 ± 0.47) than that of males (16.63 ± 1.53) at ($P \leq 0.01$).

Table 3: Effect of sex in parameters study of patients

Sex	Mean ± SE				
	T4(ng/ml)	T3(ng/ml)	TSH(ng/ml)	Ca(mg/dl)	Vit.D3(ng/ml)
Male	11.93 ± 1.07 a	1.46 ± 0.12 a	2.70 ± 0.09 a	8.30 ± 0.35 a	16.63 ± 1.53 a
Female	9.86 ± 0.43 a	1.36 ± 0.04 a	2.49 ± 0.11 a	7.48 ± 0.19 a	9.97 ± 0.47 b
LSD value	3.611 NS	0.370 NS	0.906 NS	1.597 NS	3.962 **
Means that having letters (a and b) in same column were significantly different**.** ** ($P \leq 10^{-2}$).					

In the present study we also found in table (4) that there were no significant differences in the Mean ± SE of T4, T3, Ca and vitamin D3 between age groups, but a significant differences in the Mean ± SE of TSH between age groups ($P \leq 0.05$) with LSD value (0.554).

Table 4: Effect of age groups in parameters study of patients

Age groups (year)	Mean ± SE				
	T4(ng/ml)	T3(ng/ml)	TSH(ng/ml)	Ca(mg/dl)	Vit.D3(ng/ml)
Least than 30	10.20 ± 0.57 a	1.35 ± 0.06 a	2.31 ± 0.17 b	7.55 ± 0.25 a	10.48 ± 0.63 a
30-40	9.82 ± 1.00 a	1.42 ± 0.08 a	2.40 ± 0.25 ab	7.72 ± 0.41 a	10.64 ± 1.35 a
More than 40	974 ± 0.80 a	1.36 ± 0.08 a	2.89 ± 0.11 b	7.36 ± 0.38 a	9.98 ± 0.99 a
LSD value	2.210 NS	0.226 NS	0.554 *	0.977 NS	2.425 NS
Means that having letters (a and b) in same column were significantly different**.** * ($P \leq 0.05$).					

The correlation coefficient of table (5) showed a no significant positive correlation coefficient of T4 with T3 and TSH, a significant positive correlation coefficient of T4 with Ca and vit.D3 ($P \leq 0.01$), but a no significant positive correlation coefficient of T3 with TSH and vit. D3, no significant negative correlation coefficient of T3 with Ca. Table (5) also showed no significant –ve correlation coefficient present between TSH and Ca, while no significant +ve

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correlation coefficient among TSH and vit. D3. Asignificant positive correlation coefficient was observed btween Ca and vit.D3 at (P≤0.01).

Table 5: Correlation coefficient between variables

Variables	Correlation coefficient-r	Level of sig.
T4 & T3	0.08	NS
T4 & TSH	0.11	NS
T4 & Ca	0.38	**
T4 & Vit. D	0.39	**
T3 & TSH	0.20	NS
T3 & Ca	-0.05	NS
T3 & Vit. D	0.15	NS
TSH & Ca	-0.01	NS
TSH & Vit. D	0.11	NS
Ca & Vit. D	0.55	**
** (P≤0.01), NS: Non-Significant.		

Figure (1) showed that the highest level of T4 was in patients with D3 defitiency without osteoporosis, while the lowest level of this hormone was in patients with D3 defitiency with osteoporosis.

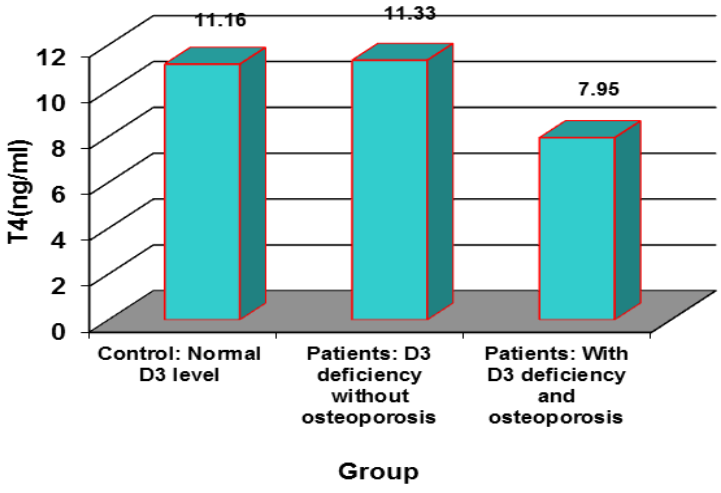

Figure 1: comparision between different groups in T4

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Figure (1) showed that the highest level of T3 was in patients with D3 defitiency without osteoporosis, while the lowest level of T3 was in patients with D3 defitiency and osteoporosis.

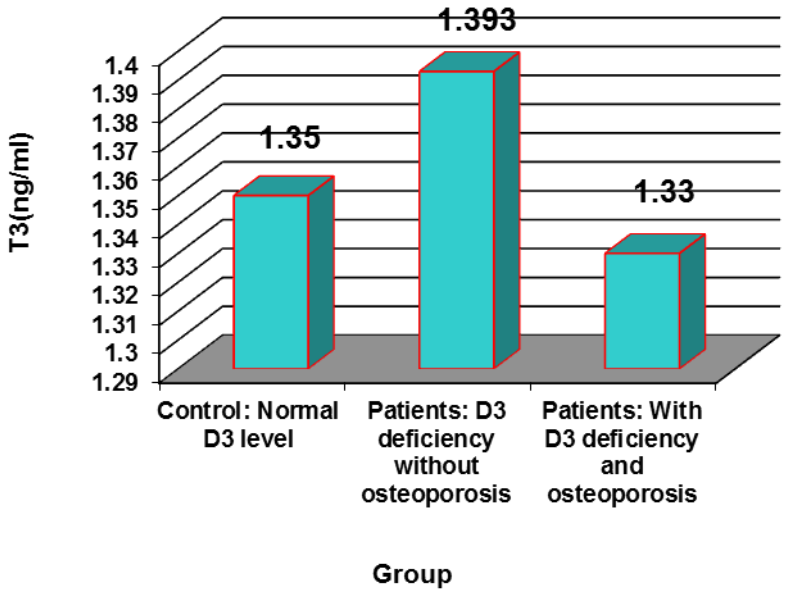

Figure 2: Comparision between different groups in T3

In Figure (3) the highest level of TSH was in patients with D3 defitiency without osteoporosis, while the lowest level was in patients with D3 defitiency and osteoporosis.

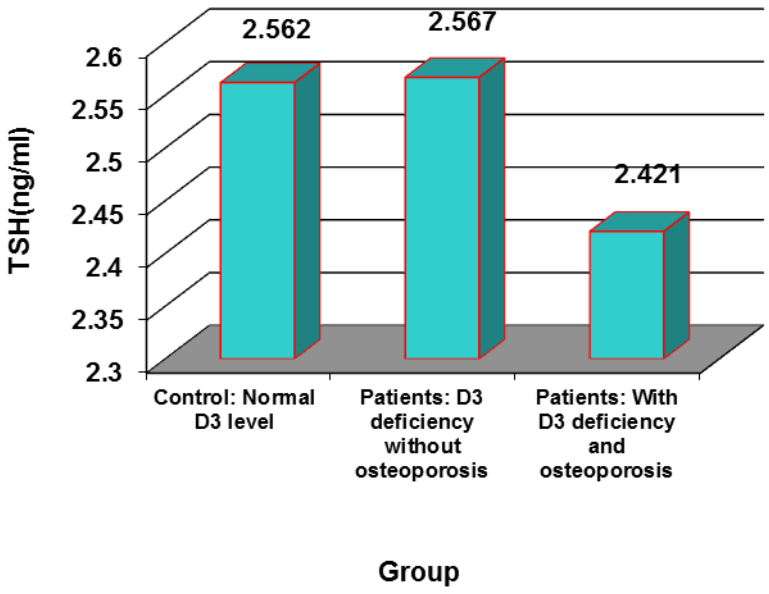

Figure 3: Comparision between different groups in TSH

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Our results in figure (4) demonstrated that the highest level of Ca was in control group, while the lowest level of Ca was in patients with D3 defitiency and osteoporosis.

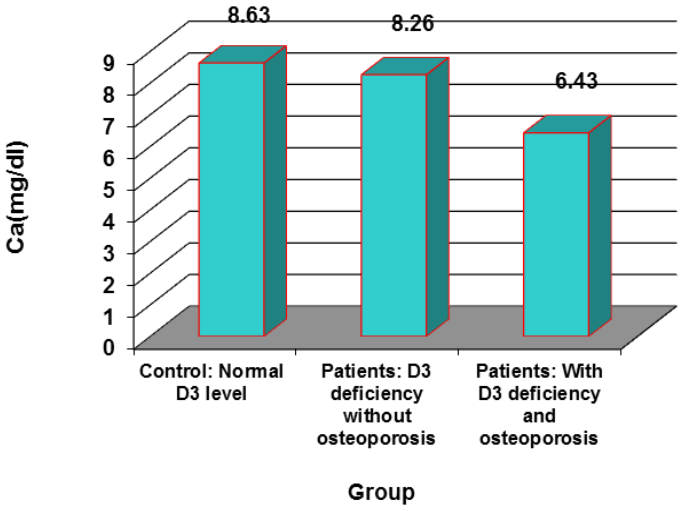

Figure 4: Comparision between different groups in Ca

Our results also showed that the highest level of vit.D3 was in control group, while the lowest level of D3 was in patients with D3 defitiency and osteoporosis (Figure 5).

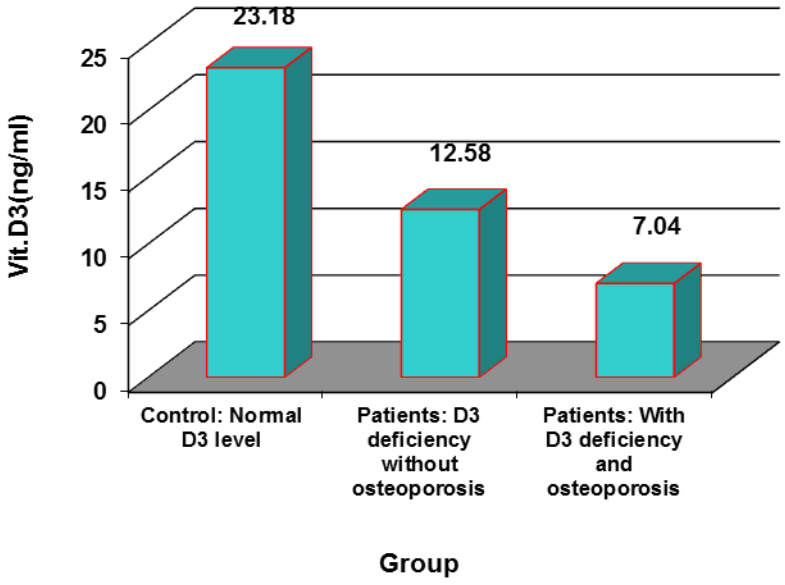

Figure 5: Comparision between different groups in vit.D3

DISCUSSION

D3 is a steroid (fat soluble) vitamin, produced in the skin (Makariou *et al*.,2011) . D3 receptors found in most tissues in the body and has many roles especially in the regulation of calcium and phosphorous homeostasis. Recently, many research suggest that D3 deficiency

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plays a role in many diseases such as autoimmune disorders, many cancers, heart diseases and subsequently may cause death (Plum *et al*.,2010). Lowering in levels of vitamin D also associated with thyroid diseases of autoimmune source (Kmiec and Sworczak, 2015). There are some of researchers that doing an examination for the prevalence of deficiency in vitamin D in Iraqi populations while the current study aimed to examine the association of Vitamin-D3 levels with Ca ions, hypo and also hyper- thyroidism in Iraq mainly Baghdad city. For this reason we are doing this study to estimate the levels of D3- vitamin in patients with hypo and hyper-thyroidism compared with healthy people that did not suffering from any symptoms of thyroid diseases.

The results in table (3) explained a significant lowering in serum vit D levels in females than in controls and patients of males that similar to author (Mohammed *et.al*.,2013). Our results was in disagreement with the study of (Elsammak *et al*.,2011; Lippi *et al*.,2012) which find a no significant differences between males and females in serum levels of vitamin D. another significant differences between males and females in the level of vitamin D were found in Tehran city in the study of Hashemipour *et.al*.(2004). Our results are in agreement with the results of Al-Jurayyan *et. al*., (2002) who find that the levels of vitamin D in the serum are significantly highly decreased in females compared with males. In Iraqi patients, levels of vitamin D was non significantly lower in old age persons than in young for men and women, this result is in agreement with the result of study in Japan (Yamashita *et al*., 2001). The differences between these studies may be related to different patients that selected, ammount of vitamin D in die and sunlight exposure. Furthermore, Table (1) showed that the Mean ± SE values of T4 lowered significantly in patients suffering from D3 deficiency and osteoporosis than Mean ± SE of control group and patients with D3 deficiency without osteoporosis ($P \leq 0.01$). The table also showed no significant difference between the three categories in the Mean ± SE of T3, Mean ± SE of TSH was different significantly between the three groups. Another findings in our study associated with serum calcium level that is lowered in patients with D3 deficiency and osteoporosis and also decreased in women than in men.

Serum levels of D vitamin and Ca shows a positive correlation through a comparision with T4 and T4, this result is in agreement with the result of Kivity *et al*.,(2011) who found a deficieny in vitamin D between patients with Hashimoto's about (92%) was higher than this level in healthy persons (63%). The reason for these results (hypothyroidism patients with low concentration of vitamin D) may related to reduced absorption of this vitamin from small intestine or the body did not activate vitamin D. Other research have demonstrated that persons with Graves's disease have a low Vitamin D levels (Yasuda *et al*.,2012). Because thyroid hormone is a steroid hormone and vitamin D3 is a fat soluble vitamin thus, they are both bind to the same receptors which named steroid hormone receptors. In addition, many recent studies explain the relationship between vitamin D and different autoimmune diseases (Naderi *et al*.,2008). Taking up of vitamin D supplements preventing the development of many kinds of autoimmune diseases (Baeke *et al*.,2010). Many studies have demonstrated a role of this vitamin in Graves Disease, either vitamin D related to polymorphisms in genes such as vit.- D receptor (VDR) gene and vitamin D binding protein gene are associated with Graves Disease or the deficiency in vitamin D cause a modulation in Graves' hyperthyroidism induced by thyrotropin receptor immunization in BALB/c mice or analog of

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vitamin D inhibits inflammatory responses in human cells of thyroid gland and T cells (Rotandi and Chiovato, 2011).

THE CONCLUSION

Our results indicated that Iraqi patients with hypothyroidism suffering from deficiency in vitamin D3 and Ca. Thre is a positive correlation between serum vitamin D and Calcium ions with T4 levels. Screening for the deficiency in vitamin D and Ca is recommended for all Iraqi patients suffering from to avoid osteoporosis if the deficiency continued.

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