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CORRELATION BETWEEN VITAMIN D LEVEL AND BONE MINERAL DENSITY IN EGYPTIAN PATIENTS WITH SECONDARY HYPERPARATHYROIDISM

By

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ABSTRACT

Background: There is still limited data on the association between 25-hydroxyvitamin D, parathyroid hormone (PTH) and bone mineral density (BMD) in Egypt.

Objective: To correlate between vitamin D level and bone mineral density in Egyptian patients with secondary hyperparathyroidism.

Patients and Methods: Fifty female patients with secondary hyperparathyroidism recruited for the study from Rheumatology and Rehabilitation Outpatient Clinic of Al-Azhar University Hospitals, in addition to another fifty age matched control group healthy individuals after an informed consent from all subjects starting from November 2019 till may 2020. In this study, we measure parathormone hormone level, 25-Hydroxyvitamin D, serum calcium (total and ionized). Assessment of bone mineral density measured by dual-energy x-ray absorptiometry at lumbar spine, left proximal femur, and left distal forearm.

Results: Lt Forearm BMD was significantly lower in the 50 women with secondary hyperparathyroidism than in the 50 with normal PTH levels. The mean t-score ± SD was -1.2 ± 0.93, and 0.5 ± 1.37 respectively. Thirty five (70%) was vit D deficient with mean t-score of the Lt Forearm -1.6 ± 1.48 (osteopenia), and fifteen (30%) was vit D insufficient with normal t-score of all bone parameters in this group.

Conclusion: Our study revealed that positive correlation and significance between Vit D with serum calcium (total and ionized), BMD and t score (AP spine, Lt Femur, and Lt forearm), while negative correlation and significance between BMD (AP spine, Lt femur, and Lt forearm) with PTH, and negative correlation and significance between vit D with PTH.

Keywords: Secondary hyperparathyroidism, parathyroid hormone, vitamin D, bone mineral density.

INTRODUCTION

Vitamin D is a steroid hormone responsible for maintaining calcium and phosphate homeostasis. Humans endogenously synthesize vitamin D in the skin upon exposure to ultraviolet B radiation and exogenously derive vitamin D from dietary sources and supplements. Once entering the circulation, vita-min D undergoes liver hydroxylation by 25-

hydroxylase enzyme and turns into 25-hydroxyvitamin D (25(OH)D), which is generally measured for assessment of vitamin D status.25(OH)D is then converted by 1α-hydroxylase enzyme in the kidney into the active from of 1,25-dihydroxyvitamin D(1,25(OH)2D) that exerts physiological functions by promoting intestinal calcium and phosphate absorption, renal tubular reabsorption of calcium, and bone resorption and formation *(Charoenngam et al., 2019)*.

Deficiency of vitamin D was linked to secondary hyperparathyroidism and bone loss, with decreased BMD and increased risk of osteoporosis *(Ebeling, 2014)*.

The general consensus on vitamin D deficiency suggests that deficiency exists when serum 25-hydroxyvitamin D levels are less than 50nmol/L (20 ng/mL), with insufficiency in the range from 50nmol/L to 75nmol/L, and sufficiency above 75nmol/L (30 ng/mL).Vitamin D status is measured in the blood using 25-Hydroxyvitamin D measurements. Keeping recommended vitamin D levels is essential for bone health. Sources of vitamin D include adequate sunlight exposure, and adequate dietary intake of vitamin D. Supplements should be used where there is inadequate amount of vitamin D *(Holick et al., 2011)*.

The present work aimed to correlate between vitamin D level and bone mineral density in Egyptian patients with secondary hyperparathyroidism, and to determine in the Egyptian population link between the vitamin D deficiency and osteoporosis.

PATIENTS AND METHODS

The ethical approval was obtained from the hospital ethical research committee. Fifty female patients with secondary hyperparathyroidism recruited for the study from Rheumatology and Rehabilitation Outpatient Clinic of Al-Azhar University Hospitals, in addition to another fifty age matched control group, after obtaining informed consents from all subjects. This work started from November 2019 till May 2020. We measured parathormone hormone level, 25-Hydroxyvitamin D, serum calcium (total and ionized). Assessment of bone mineral density measured by dual-energy x-ray absorptiometry at lumbar spine, left proximal femur and left distal forearm. Densitometer was calibrated daily, and quality assurance was performed monthly.

Inclusion criteria:

This included an existing diagnosis of elevated parathormone hormone for secondary hyperparathyroidism.

Exclusion criteria:

Primary hyperparathyroidism diagnosis with elevated serum calcium and unrestrained levels with PTH.

- Patients with conditions associated with malabsorption of vitamin D.
- Patients taking any medication(s) that can adversely affect bone metabolism and thus lead to decreased BMD by causing deficiency of vitamin D.
- Patients with a 50 ml / min creatinine clearance. Below this stage, 25(OH) D to 1,25-hydroxyvitamin D has impaired hydroxylation.

- Patients who received vitamin D supplements, and mothers who were pregnant or lactating.

All patients were subjected to the following:

1. Careful history taking.
2. Musculoskeletal examination.
3. The following laboratory investigations were done for all cases:
   a. Parathormone hormone level by chemoillumicience technique and serum calcium (total and ionized).
   b. 25-Hydroxyvitamin D Level of serum 25-Hydroxycholecalciferol [25-(OH)D] by Chemoillumicience technique.

4. Bone Quantity Assessment was measured by dual-energy X-ray absorptiometry at lumbar spine, left proximal femur and left distal forearm. Densitometer was calibrated daily, and quality assurance was performed monthly.

Type of apparatus: GE Medical System (GE-Lunar Prodigy Primo) Madison, WI, USA Brand: GE-Lunar Model: Prodigy.

Bone mineral density (BMD) or bone mass is one of the predictors of fracture. With the availability of high precision bone densitometers, the World Health Organization has established criteria for diagnosing osteoporosis.

The diagnostic score (t-score) is related to bone mass in young healthy women. Bone mineral density (BMD) above –1 SD (standard deviation) is normal, BMD between –1 and –2.5 SD is assigned to osteopenia, while below –2.5 SD is the diagnostic criterion for osteoporosis (T-score) BMD is preferably measured by dual energy X-ray absorptiometry (DXA).

Statistical analysis:

Data were analyzed using Statistical program for Social Science (SPSS) version 26.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

RESULTS

Regarding patients' characteristics, there were statistically significant differences between groups according to Age, PTH, S.Ca T, Vit D, BMD and T score (AP spine, Lt femur, Lt forearm) **(Table 1)**.

Table (1): Comparison between cases and controls regarding patients' characteristics

Groups / Variables	Cases (n = 50)	Controls (n=50)	P-value
• Age			0.002
Mean ± SD.	33.6 ±10.12	39.7 ± 7.45	
• PTH			<0.001
Mean ± SD.	148.1 ±109.65	53.0 ± 13.69	
• S.Ca T			<0.001
Mean ± SD.	8.4 ± 0.84	9.2 ± 0.66	
• S.Ca I			0.225
Mean ± SD.	1.5 ± 0.74	1.4 ± 0.41	
• Vit D			<0.001
Mean ± SD.	13.2 ± 8.14	42.3 ± 11.25	
• AP spine			
BMD Mean ± SD.	1.1 ± 0.17	1.2 ± 0.15	<0.001
T score Mean ± SD.	-0.8 ± 1.33	0.3 ± 1.07	<0.001
• Lt femur			
BMD Mean ± SD.	0.9 ± 0.17	1.1 ± 0.10	<0.001
T score Mean ± SD.	-0.5 ± 1.45	0.6 ± 0.73	<0.001
• Lt Forearm			
BMD Mean ± SD.	0.6 ± 0.10	0.7 ± 0.11	<0.001
T score Mean ± SD.	-1.2 ± 1.57	0.5 ± 1.37	<0.001

Regarding to relation between age, laboratory, bone parameters and Vit D groups in cases, there were statistically significant differences between age, laboratory, bone parameters and Vit D groups in cases according to age , PTH , s.Ca T , BMD and t score (AP spine , Lt femur , Lt forearm) **(Table 2)**.

Table (2): Relation between age, laboratory, bone parameters and Vit D groups in Cases

Vit D groups / Variables	Deficiency (<20) (n=35)	Insufficiency (20 – 30) (n=15)	P-value
• Age			0.022
Mean ± SD.	35.8 ± 9.22	28.4 ± 10.58	
• PTH			0.007
Mean ± SD.	174.1 ± 121.27	85.7 ± 16.10	
• S.Ca T			0.004
Mean ± SD.	8.2 ± 0.81	8.9 ± 0.66	
• S.Ca I			0.181
Mean ± SD.	1.5 ± 0.76	1.5 ± 0.72	
• AP spine			0.047
BMD Mean ± SD.	1.1 ± 0.17	1.2 ± 0.13	0.021
T score Mean ± SD.	-1.1 ± 1.40	-0.1 ± 0.87	
• Lt femur			0.032
BMD Mean ± SD.	0.9 ± 0.14	1.0 ± 0.20	0.038
T score Mean ± SD.	-0.8 ± 1.23	0.2 ± 1.69	
• Lt Forearm			0.044
BMD Mean ± SD.	0.6 ± 0.10	0.6 ± 0.09	0.015
T score Mean ± SD.	-1.6 ± 1.48	-0.4 ± 1.54	

Regarding to relation between age, laboratory parameters and AP spine t score in cases, there were statistically significant differences between age, laboratory parameters and AP spine t score in Cases according to PTH, S.Ca T and Vit D and no statistically significant difference between age, laboratory parameters and AP spine t score in cases according to age and s.Ca I (Table 3).

Table (3): Relation between age, laboratory parameters and AP spine t score in Cases

AP spine t score / Variables	Normal (n=30)	Osteopenia (n=15)	Osteoporosis (n=5)	P-value
• Age				0.392
Mean ± SD.	32.3 ± 10.13	35.7 ± 10.71	36.0 ± 8.51	
• PTH				0.002
Mean ± SD.	120.9 ± 93.89	145.8 ±85.72	323.0±120.36	
• S.Ca T				0.033
Mean ± SD.	8.6 ± 0.84	8.3 ± 0.79	7.6 ± 0.33	
• S.Ca I				0.157
Mean ± SD.	1.6 ± 0.84	1.3 ± 0.42	1.3 ± 0.81	
• Vit D				0.005
Mean ± SD.	15.4 ± 8.59	11.5 ± 6.13	4.9 ± 2.76	

Regarding to relation between age, laboratory parameters and Lt femur t score in cases, there were statistically significant differences between age, laboratory parameters and Lt femur t score in cases according to age , PTH , s.Ca I and shows no statistically significant difference between age, laboratory parameters and Lt Femur t score in Cases according to S.Ca T, Vit D **(Table 4)**.

Table (4): Relation between age, laboratory parameters and Lt Femur t score in Cases

Lt Femur t score / Variables	Normal (n=28)	Osteopenia (n=19)	Osteoporosis (n=3)	P-value
• Age				<0.001
Mean ± SD.	37.2 ± 8.58	26.5 ± 2.02	44.0 ± 1.73	
• PTH				0.003
Mean ± SD.	104.9 ±61.44	190.0±126.19	300.0±161.00	
• S.Ca T				0.072
Mean ± SD.	8.6 ± 0.86	8.1 ± 7.4	7.8 ± 0.54	
• S.Ca I				0.033
Mean ± SD.	1.6 ± 0.75	1.4 ± 0.74	0.9 ± 0.19	
• Vit D				0.112
Mean ± SD.	14.6 ± 8.14	12.3 ± 8.18	5.8 ± 3.32	

Regarding to relation between age, laboratory parameters and Lt Forearm t score in Cases, there was statistically significant difference between age, laboratory parameters and Lt Forearm t score in Cases according to Age , PTH , S.Ca T&I ,Vit D **(Table 5)**.

Table (5): Relation between age, laboratory parameters and Lt Forearm t score in Cases

Lt Forearm t score / Variables	Normal (n=22)	Osteopenia (n=17)	Osteoporosis (n=11)	P-value
• Age				0.027
Mean ± SD.	36.9 ± 8.57	28.9 ± 10.02	34.2 ± 11.27	
• PTH				<0.001
Mean ± SD.	99.4 ± 57.55	117.9 ±79.33	296.5±107.34	
• S.Ca T				<0.001
Mean ± SD.	8.9 ± 0.65	7.9 ± 0.76	7.9 ± 0.54	
• S.Ca I				<0.001
Mean ± SD.	1.7 ± 0.80	1.6 ± 0.75	0.9 ± 0.09	
• Vit D				<0.001
Mean ± SD.	16.1 ± 7.68	14.1 ± 8.37	5.9 ± 3.25	

Regarding to correlation between laboratory parameters and AP spine BMD in cases, there was negative correlation and significance between AP spine BMD in Cases with PTH while, positive correlation and significance between AP spine BMD in cases with s.Ca T&I and Vit D **(Table 6)**.

Table (6): Correlation between laboratory parameters and AP spine BMD in Cases

AP spine BMD / Lab. Findings	r	P-value
PTH	-0.448	0.001
SCa.T	0.439	0.001
SCa.I	0.307	0.029
Vit D	0.425	0.002

Regarding to correlation between laboratory parameters and Lt femur BMD in cases, there were negative correlation and significance between Lt femur BMD in cases with PTH while, positive correlation and significance between Lt femur BMD in cases with s.Ca T&I and Vit D **(Table 7)**.

Table (7): Correlation between laboratory parameters and Lt Femur BMD in Cases

Lt Femur BMD / Lab. Findings	r_s	P-value
PTH	- 0.668	<0.001
SCa.T	0.507	<0.001
SCa.I	0.454	0.001
Vit D	0.491	<0.001

Regarding to correlation between laboratory parameters and Lt forearm BMD in Cases, there were negative correlation and significance between Lt forearm BMD in cases with PTH, while positive correlation and significance between Lt forearm BMD in cases with S.Ca T&I and Vit D **(Table 8)**.

Table (8): Correlation between laboratory parameters and Lt Forearm BMD in Cases

Lab. Findings \ Lt Forearm BMD	r	P-value
PTH	**-0.600**	**<0.001**
SCa.T	**0.441**	**0.001**
SCa.I	**0.423**	**0.002**
Vit D	0.413	0.003

Regarding to correlation between age, laboratory parameters and Vit D in Cases, there was negative correlation and significance between Vit D in Cases with PTH While, positive correlation and significance between Vit D in cases with s.Ca T&I and t score (AP spine, Lt femur, Lt forearm) **(Table 9)**.

Table (9): Correlation between laboratory, bone parameters and Vit D in Cases

Variables \ Vit D	r_s	P-value
Age	**-0.187**	**0.189**
PTH	**-0.552**	**<0.001**
SCa.T	**0.454**	**0.001**
SCa.I	**0.366**	**0.008**
AP spine t score	**0.465**	**0.001**
Lt Femur t score	**0.496**	**<0.001**
Lt Forearm t score	**0.561**	**<0.001**

DISCUSSION

In our retrospective cohort study on fifty Egyptian patients diagnosed as secondary hyperparathyroidism with a mean age of 33.6 ± 10.12 years, mean PTH 148.1 ± 109.65 and mean vit D 13.2 ± 8.14. Another fifty age matched control group with a mean age of 39.7 ± 7.45 years, mean PTH 53.0 ± 13.69 and mean vit D 42.3 ± 11.25 were included in the study. The comparison between cases and controls regarding patients' characteristics revealed that the Lt forearm is the most affected site in case group with mean t-score -1.2 ± 1.57, but the other bone parameters in both groups were normal. Thirty five patients were vit D deficient with mean t-score of the Lt forearm -1.6 ± 1.48 (osteopenia), mean t-score of the AP spine -1.1 ± 1.40 (osteopenia) and fifteen were vit D insufficient with normal t-score of all bone parameters in this group.

Normal AP spine t- score was in thirty patients, osteopenia in fifteen with mean values for serum 25(OH) D and PTH levels were 11.5 ± 6.13 ng/ml and 145.8 ±85.72 pg/ml, respectively, and osteoporosis in five with mean values for serum 25(OH) D and PTH levels were 4.9 ± 2.76 ng/ml and 323.0±120.36 pg/ml, respectively.

Normal Lt femur t- score in twenty eight patients, osteopenia in nineteen with mean values for serum 25(OH) D and PTH levels were 12.3 ± 8.18 ng/ml and 190.0±126.19 pg/ml, respectively, and osteoporosis in Three (3) with mean values for serum 25(OH) D and PTH

levels were 5.8 ± 3.32 ng/ml and 300.0±161.00 pg/ml, respectively.

Normal Lt Forearm T- score in twenty two patients, osteopenia in seventeen with mean values for serum 25(OH) D and PTH levels were 14.1 ± 8.37 ng/ml and 117.9 ±79.33 pg/ml, respectively, and osteoporosis in eleven with mean values for serum 25(OH) D and PTH levels were 5.9 ± 3.25ng/ml and 296.5±107.34 pg/ml, respectively.

Our study showed a statistically significant difference between cases and controls according to Age, PTH, Vit D, BMD and T score (AP spine, Lt Femur, Lt forearm). This result agreed with that of *Mendes (2019)* where age was positively correlated with BMD at the femur but not BMD at the lumbar spine. There were no significant differences between age groups in serum 25(OH) D, plasma PTH, and serum calcium. *Kota (2013)* at the hip; PTH levels and age were found to be significant predictors of BMD.at the lumbar spine; PTH levels and age were independently predictive of BMD.

Our study shows statistically significant difference between Vit D groups in Cases according to PTH. Negative correlation and significance between vit D in cases with PTH. This result agreed with that of *Mendes (2019)* who reported that 25 (OH) D concentrations being inversely correlated with PTH concentrations in healthy adult women. Additionally, 10.4% of participants had secondary hyperparathyroidism, with a higher prevalence amongst those with deficient and insufficient vitamin D status. *El Badawy (2014)* showed an inverse correlation between 25(OH)D and PTH. *Vučeljić et al., (2012)* showed a very significant inverse correlation between 25(OH) D and PTH was established by BMD at lumbar spine. In patients with 25(OH) D insufficiencies, elevated PTH varied individually, but it was most often increased if 25(OH) D was equal or lower than 37.6 nmol/L. *Kota (2013)* reported that a negative correlation between PTH and 25(OH) D at serum 25(OH) D concentrations <30 ng/ml.

Our study showed a statistically significant difference between Vit D groups in cases according to BMD & t score (AP spine, Lt femur, Lt forearm). This result agreed with that of Mendes (2019) where no significant correlations between lumbar spine (L1–L4) and femur bone parameter measurements, determined by DXA, and 25(OH) D concentrations for women living in Brazil. *Kota (2013)* showed no statistically significant associations between serum 25(OH) D concentrations and BMD at the hip and lumbar spine. No direct relationship between serum 25(OH) D levels and BMD was observed. *Vučeljić et al., (2012)* showed very significant inverse correlations between 25(OH) D and PTH was established by BMD at lumbar spine. *Alkhenizan (2017)* reported that no significant correlation between spine or total femoral BMD and serum 25(OH) D. No correlation has been found between vitamin D deficiency and reduced bone mineral density in any age group, in males or females.

Our study showed a statistically significant difference between AP spine t score in cases according to PTH, Vit D. Negative significant correlation between AP spines BMD in cases with PTH.

While, positive correlation and significant between AP spine BMD in Cases with Vit D. Positive significant correlation between Vit D in cases with t score (AP spine). This result agreed with that of *Mendes (2019)* where no significant correlations between lumbar spine (L1–L4) bone parameter measurements, determined by DXA, and 25(OH)D concentrations for women living in Brazil. *Kota (2013)* showed no statistically significant associations between serum 25(OH) D concentrations and BMD at lumbar spine. *Alkhenizan (2017)* reported that no significant correlation between spine BMD and serum 25(OH) D.

Our study showed a statistically significant difference between Lt femur t score in cases according to PTH. Negative significant correlation between Lt femur BMD in cases with PTH, while positive significant correlation between Lt femur BMD in cases with vit D, and positive significant correlation between vit D in cases with t score (Lt femur). This result agreed with that of *Mendes (2019)* where no significant correlations between femur bone parameter measurements, determined by DXA, and 25(OH) D concentrations for women living in Brazil. *Kota (2013)* at the hip found that PTH levels were significant predictors of BMD. *Di Monaco (2016)* showed that PTH levels in the presence of severe vitamin D deficiency were significantly associated with femoral BMD in women. *Amaozugan (2011)* did not find any significant associations between PTH status and hip BMD.

Our study showed no statistically significant difference between Lt femur t score in cases according to Vit D. This result agreed with that of *Alkhenizan (2017)* who reported that no significant correlation between total femoral BMD and serum 25(OH) D. *Kota (2013)* showed no statistically significant associations between serum 25(OH) D concentrations and BMD at the hip. *Di Monaco (2016)* showed the presence of secondary hyperparathyroidism which was significantly associated with a femoral neck t-score lower than -2.5.

Our study showed a statistically significant difference between Lt forearm t score in cases with PTH and Vit D. Negative significant correlation between Lt forearm BMD in cases with PTH, while positive significant correlation between Lt forearm BMD in cases with Vit D, and positive significant correlation between Vit D in cases with t score (Lt forearm).

Our study showed negative significant correlation between Vit D in cases with PTH, while positive significant correlation between Vit D in cases with s.Ca T & I. This result agreed with that of *El Badawy (2014)* who showed an inverse correlation between 25(OH)D and PTH. Low calcium and ionized calcium are significantly correlated with vitamin D levels. *López-Ramiro et al., (2016)* showed an inverse association between serum 25OHD and PTH. *Baroncini (2018)* showed a negative correlation between PTH levels and 25(OH) D.

CONCLUSION

Positive significant correlation between Vit D with serum calcium (Total and Ionized), BMD and t score (AP spine, Lt femur, Lt forearm), negative significant correlation between BMD (AP spine, Lt femur, Lt forearm) with PTH, and

negative significant correlation between vit D with PTH.

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العلاقة بين مستوى فيتامين (د) وكثافة العظام عند المرضى المصريين المصابين بفرط نشاط الغدة الجار درقية الثانوى

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خلفيـــة البحـــث: لاتـــزال هنـــاك بيانـــات محـــدودة عـــن الارتبـــاط بـــين 25-هيدروكســـى فيتامين د، وهرمون الغدة الجار درقية وكثافة العظام في مصر.

الهـــدف مـــن البحـــث: الـــربط بـــين مســـتوى فيتـــامين (د) وكثافـــة العظـــام فـــى المرضـــى المصريين المصابين بفرط نشاط الغدة الجار درقية الثانوى.

المريضـــات وطـــرق البحـــث: تـــم دراســـة خمســـون مريضـــة بفـــرط نشـــاط الغـــدة الجـــار درقيـــة الثـــانوى مـــن عيـــادة الرومـــاتيزم والتأهيـــل الخارجيـــة بمستشـــفيات جامعـــة الازهـــر ، بالاضـــافة الـــى خمســـين اخـــرى مطابقـــة لمجموعـــة مراقبـــة مطابقـــة للنســـاء الصـــحيحات ، بعـــد اخـــذ موافقـــة مســـتنيرة مـــن جميـــع الحـــالات بـــدءا مـــن نـــوفمبر 2019 حتـــي مـــايو 2020ز وفـــى هـــذه الدراســـة تـــم قيـــاس مســـتوى هرمـــون الغـــدة الجـــار درقيـــة، 25-هيدروكســـى فيتـــامين د، والكالســـيوم فـــى الـــدم (المجمـــوع والمـــؤين). وقيمـــت كثافـــة العظـــام بجهـــاز هشاشـــة العظـــام علـــى الفقـــرات القطنيـــة وعظـــم الفخـــذ الأيســـر والســـاعد الأيسر.

نتـــائج البحـــث: كانـــت كثافـــة عظـــام الســـاعد الايســـر أقـــل بكثيـــر فـــى النســـاء اللاتـــى تعـــانين مـــن فـــرط نشـــاط الغـــدة الجـــار درقيـــة الثـــانوى ممـــا كانـــت عليـــه فـــي الصـــحيحات، وفيتـــامين د كان ناقصا في 70 % من المريضات.

الاســـتنتاج: هنـــاك ارتباطـــا ايجابيـــا بـــين فيتـــامين د مـــع الكالســـيوم فـــى الـــدم وكثافـــة العظـــام ودرجـــة تـــى فـــى (الفقـــرات القطنيـــة، الفخـــذ الايســـر، الســـاعد الايســـر) مـــع هرمـــون الغـــدة الجار درقية، وارتباط سلبى بين فيتامين د مع هرمون الغدة الجار درقية.