

## THE RELATIONSHIP BETWEEN VITAMIN D AND ULTRASONOGRAPHIC AND CYTOLOGICAL FINDINGS OF THYROID NODULES

### TİROİD NODULLERİNDE ULTRASONOGRAFİK VE SİTOLOJİK BULGULAR İLE VİTAMİN D ARASINDAKİ İLİŞKİNİN DEĞERLENDİRİLMESİ

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#### ABSTRACT

**PURPOSE:** There are reported associations between Vitamin D and some cancers, but the relationship between vitamin D and thyroid cancers has not been fully evaluated. The aim of this study was to evaluate the relationship between vitamin D levels and ultrasonographic, cytologic and postoperative pathologic data of the thyroid nodules.

**METHODS:** The study included 225 patients who underwent thyroidectomy after thyroid fine needle aspiration biopsy (FNAB) due to the presence of thyroid nodules. The preoperative ultrasonographic findings, the cytopathologic results obtained after FNAB and thyroidectomy operation and serum vitamin D levels were recorded. The relationship between vitamin D levels and ultrasonographic and cytologic findings of thyroid nodules was analyzed.

**RESULTS:** There was no statistically significant relationship between vitamin D and ultrasonographic and histopathologic results of thyroid nodules.

**CONCLUSIONS:** Our results showed that there was no relationship between Vitamin D and ultrasonographic characteristics, biopsy and surgery results of thyroid nodules. However, when we retrospectively examined our results, we observed that very few cases with malignancy were found to be benign in the preoperative FNAB evaluation. These patients had low vitamin D levels and a nodule size of more than 2.5 cm. We think that more extensive studies are needed to assess the association between malignancy and large thyroid nodules associated with vitamin D deficiencies.

**Keywords:** Vitamin D, Thyroid Nodule, Ultrasonography, Cytology

#### ÖZET

**AMAÇ:** Vitamin D ile bazı kanserler arasında bir ilişki olduğu bildirilmiş, ancak vitamin D ile tiroid kanserleri arasındaki ilişki tam olarak değerlendirilmemiştir. Bu çalışmanın amacı, D vitamini düzeyleri ile tiroid nodüllerinin ultrasonografik, sitolojik ve postoperatif patolojik verileri arasındaki ilişkiyi değerlendirmektir.

**YÖNTEM:** Çalışmamıza tiroid nodül varlığı nedeniyle tiroid ince iğne aspirasyon biyopsisi (TİİAB) sonrası tiroidektomi operasyonu uygulanan 225 hasta dahil edildi. Çalışmaya dahil edilen hastaların preoperatif ultrasonografik bulguları, TİİAB ve tiroidektomi operasyonu sonrası elde edilen sitopatolojik sonuçları ile serum vitamin D düzeyleri kaydedildi. Vitamin D düzeyleri ile tiroid nodüllerinin ultrasonografik ve sitolojik bulguları arasındaki ilişki analiz edildi.

**SONUÇLAR:** Vitamin D ile tiroid nodüllerinin ultrasonografik özellikleri, TİİAB ve cerrahi sonrası histopatolojik sonuçları arasında istatistiksel anlamlı ilişki saptanmadı.

**TARTIŞMA:** Sonuçlarımız D vitamini ile tiroid nodüllerinin ultrasonografik özellikleri, TİİAB ve cerrahi sonuçları arasında ilişki olmadığını göstermiştir. Bununla birlikte, sonuçlarımızı geriye dönük olarak incelediğimizde, operasyon sonrası malign saptanan çok az sayıda vakanın preoperatif TİİAB değerlendirmesinde benign saptandığını gözlemledik. Bu olgular vitamin D düzeyleri düşük ve nodül boyutu 2,5 cm'nin üzerinde olan olgulardı. Vitamin D eksikliğinin eşlik ettiği büyük tiroid nodülleri ile malignite arasındaki ilişkiyi değerlendirmek için daha kapsamlı çalışmalara ihtiyaç olduğunu düşünüyoruz.

**Anahtar kelimeler:** Vitamin D, Tiroid Nodülü, Ultrasonografi, Sitoloji

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## INTRODUCTION

Thyroid nodule is a common pathology. After the detection of a thyroid nodule, it is important to determine the nature of it and plan how to treat and follow it up. Prevalence of the nodules in the general population is around 4-10%, but by autopsy surveys the rates increases by 37 to 57% (1-3). In radiological surveys using thyroid ultrasonography (USG), 20-76% of adults were found to have thyroid nodules (4). Incidence of the nodules markedly increases in iodine deficient regions as in our country. Although higher prevalence of thyroid nodules were expected in our country where serious or moderate iodine deficiency were seen in last decades, in Turkey according to different screening studies, sonographic prevalence of the nodules in people aged 18-65 years was 23.5%, it was 37.4 after 65 years (5,6). Despite its relative frequency, studies have shown that only 5-15% of thyroid nodules demonstrate histologically proven malignancy and thyroid cancer comprises 0.5-1% of all malignancies in adults and accounts for 3% of childhood cancers (7-9). Although the rate seems low, the early diagnosis of these cancers is very important because of their slow progression and patients longevity due to early treatment. After detection, thyroid nodule should be evaluated with USG. Numerous studies have attempted to define ultrasound features that may predict benignity and malignancy (10-12). For cytological interpretation, fine needle aspiration biopsy (FNAB) is an established diagnostic modality in the evaluation of thyroid nodules.

The essential role of Vitamin D (Vit D) in bone and calcium metabolism is well known (13). Besides, it is clear that Vit D has additional physiological functions. There are studies about vitamin D deficiency being a risk factor for hypertension, type 1 and 2 diabetes mellitus, cardiovascular disease, and various cancers (14-22). However the association between Vit D levels and thyroid cancer is unknown.

In our study we aimed to compare Vit D levels and ultrasonographic and the cytologic results of the patients exposed to FNAB and surgery, and find if Vit D levels affect them.

## MATERIAL AND METHODS

**Patients:** This retrospective study was approved by ethics board of Kafkas University Faculty of Medicine (Approval number: 10.12.2014 / 112). Informed consent was not required. A total of 225 patients with thyroid nodules aged between 17-83 years [189 female (84%), 36 male (16%)] who admitted to outpatient Clinics of Endocrinology and Metabolism and also Internal Medicine of Kafkas University from October 2012 to October 2014 and had thyroid USG and FNAB were included in this study. Subjects without complete information or taking medications that affected their thyroid function, such as oral contraceptives, estrogen, glucocorticoids and iodine and women having doubt of pregnancy were excluded.

## Laboratory Measurements:

Free triiodothyronine (fT3), free thyroxine (fT4), thyroid stimulating hormone (TSH), Vitamin D3 (25(OH)D), thyroid autoantibodies; thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) levels of the patients were noted. fT3, fT4, TSH concentrations were determined by Access immunoassay method using Beckman Coulter DX1600 device. TPOAb and TgAb were examined by chemiluminescent immunoassay method using Cobas 4001 device. Vitamin D levels were examined by Cobas e 411 (Roche Diagnostics GmbH, Mannheim, Germany) with electrochemiluminescence protein binding assay method. Serum 25 hydroxyvitamin D (25(OH)D) levels are classified in accordance with 2011 Endocrine Society guidelines as ; 20 ng/ml and lower: vitamin D deficiency, between 20-29 ng/ml is classified as vitamin D insufficiency and above 29 ng/ml is classified as normal vitamin D level.

Euthyroidism was defined as the absence of hypo and hyperthyroidism and also without subclinical hypo and hyperthyroidism. Hypothyroidism was defined as the presence of TSH levels  $\geq 5.0$  uIU/ml and fT4 levels  $\leq 0.8$  ng/dl, hyperthyroidism was defined as the presence of TSH levels  $\leq 0.35$  uIU/ml and fT4 levels  $\geq 1.9$  ng/dl. Subclinical hypothyroidism was defined as the presence of TSH levels  $\geq 5.0$  uIU/ml and normal fT4 levels. Subclinical hyperthyroidism was defined as the presence of TSH levels  $\leq 0.35$  uIU/ml and normal fT4 levels. Patients with subclinical hypothyroidism and subclinical hyperthyroidism were excluded from study. Reference ranges of the parameters were as follows TSH: 0.34-5.6 uIU/ml, fT3: 2.5-3.9 pg/ml, fT4: 0.6-1.1 ng/ml, TPOAb  $> 34$  IU/ml positive, TgAb  $> 115$  IU/ml positive, Vit D  $< 20$  IU/ml: deficient, 20-30 IU/ml : insufficient, 30- 100 IU/ml: normal (14).

The presence of thyroid nodule(s) and size of the thyroid gland were determined by thyroid ultrasonography. As every patient with a thyroid nodule is a candidate for FNAB, in our Clinic of Endocrinology and Metabolism section one doctor performed FNABs with the guide of USG. If surgical decision was made, it was performed in our Clinic of General Surgery.

## USG:

Toshiba brand Aplio6 model using 12 MHz ultrasound probe was utilized in this study. The patient was placed in the supine position without a pillow with his or her neck in extension. Structure and size of thyroid tissue and presence of nodule were examined. The parenchymal structure (solid, cystic or mixed), size, location, number, shape, boundaries, acoustic halo, echo intensity, echo uniformity of the nodules were recorded. Three dimensions of the nodule were measured and the largest diameter was determined. Echogenicity of the nodule was named as iso-, hypo- or hyperechoic according to thyroid tissue. Calcifications were classified as rough and micro. In Doppler examination vascularisation was evaluated and classified as intranodular and peripheral.

Specific ultrasonography features of a nodule that raise suspicion for malignancy were accepted as follows: solidity, tall configuration (the anteroposterior diameter of the nodule is greater than its transvers diameter), markedly hypoechoic, microcalcifications, intranodular vascularity, irregular margin, no halo. One of these USG features was accepted suspicious.

**FNAB:**

After patient was placed in supine position, a pillow was put under the shoulders, their neck was brought to maximum extension. The neck region was cleared with iodine. No anesthetic agent was used. Aspiration was performed with a 10 cc injector mounted 22G needle once or more if necessary. Biopsy materials were evaluated in Clinic of Pathology. Preparations were examined with light microscopy after they were stained with May-Grunwald-Giemsa, Hematoxylin and eosin and covered with coating material. The occurrence of at least 6 follicle groups consisting at least 10 cells without artifacts were accepted as qualification criteria. The cytological diagnosis was given according to Bethesda system. The cytology results were stratified into following 6 categories: non-diagnostic, benign, atypia of undetermined significance, follicular neoplasm or suspicious for follicular neoplasm, potentially malignant and malignancy. Malign group consists potentially malignant and malign categories. In benign group non-diagnostic, benign, atypia of undetermined significance and follicular neoplasm or suspicious for follicular neoplasm categories were included.

**Statistical Analysis**

Calculations were performed using SPSS version PASW 18. Descriptive data on the numerical measurements obtained in this study were determined as mean, standard deviation, median, minimum, maximum, and the descriptive statistics of categorical variables were determined as number and percentage. The relationship between categorical variables have been studied with Pearson-Qui Square and Fisher Exact Qui Square, Fisher-Freeman Halton tests. Shapiro Wilk test was used for determining whether the numerical values were normally distributed. We compared the groups (consisting of two categories) in terms of the mean of numerical variables by Student t test and in terms of the median of them by Mann Whitney U test. One way ANOVA and Kruskall-Wallis tests were used to compare the mean of numerical variables of the groups (consisting more than two categories). In order to compare the methods used in the study with biopsy which is regarded as a gold standard, sensitivity, selectivity, positive predictive value and negative predictive value rates were analyzed. The relationship between numerical variables were investigated by Spearman and Pearson correlation analysis. P value of <0.05 was considered as statistically significant.

**RESULTS**

A total of 225 thyroid nodules were noted for the study. Thirty six patients (16%) were male and 189 were female (84%). Female-male ratio of the nodules was 5.2. The

average age of female patients was 48.9 ± 12.7 years and male patients was 53.6 ± 11.1 years, total age was 49.6 ± 12.6 years. The mean age was significantly higher in men than women (p=0.039).

In terms of thyroid hormone status our patients were mostly euthyroid (79.0%). Hypothyroidism rate was 20.0% and hyperthyroidism was 1.0%. The rate of the patients with TPOAb positivity was 22.9% and TgAb positivity was 22.3%. Vit D results of the patients were as follows: 172 (76.4%) of them were deficient, 27 (12.0%) of them were insufficient, 26 (11.6%) was normal. The average level of Vit D was 16.1 ± 7.6 IU/ml. In males the level was 16.2 ± 8.2 IU/ml and in females 14.4 ± 4.9 IU/ml.

There was no correlation between thyroid hormone levels and vitamin D levels. The size of thyroid nodules were between 5-61 mm (19.5 ± 10.3), in 14 cases the size of the nodules were > 4cm and in others the size were 0.5-1.5 cm. As Vit D levels decreased, thyroid nodule sizes significantly increased (p: 0.02, r: -0.299).

In **Table 1** USG characteristics of the nodules and Vit D levels were presented. When Vit D levels and characteristics of the nodules were evaluated, we did not find significant difference in Vit D levels (deficient, insufficient and normal) of the patients and the number, the structure, echogenicity, calcification, vascularity, boundaries and halo sign of the nodules.

Nodules having one of those features, which were solidity, tall configuration, markedly hypoechoic, microcalcifications, intranodular vascularity, irregular margin, no halo were listed as suspicious. Evaluation of Vit D levels and ultrasonographic results as suspicious and benign were presented in **Table 2**.

It was ultrasonographically determined that 98 (43.6%) cases were benign and 127 (56.4%) were suspicious. In deficient group 52.3% patients had suspicious and 47.7% had benign USG features. Thirty seven percent nodules in insufficient group had benign USG signs and 63% of this group had suspicious signs. In the group where normal Vit D levels were encountered, 23.1% had benign, 76.9% had suspicious USG characteristics. When the relationship between Vit D levels and malign-benign USG features were examined no relationship was found. FNAB was performed to all patients who participated in the study. During and after the procedure there were no complications. FNAB results were as follows:

56 (24.9 %)	non-diagnostic
137 (60.8 %)	benign
18 (8.0 %)	atypia of undetermined significance
6 (2.7 %)	follicular neoplasm or suspicious for follicular neoplasm
4 (1.8 %)	suspicious for malignancy
4 (1.8 %)	malignant

**Table 1. USG characteristics of the nodules**

			Vit D						p
	n	%	Deficient		Insufficient		Normal		
	n	%	n	%	n	%	n	%	
Number Single	88	39.1	26	29.6	31	35.2	31	35.2	0.209
Multi	137	60.9	48	35.0	44	32.1	45	32.9	
Structure Solid	111	49.3	60	54.0	31	27.9	20	18.1	0.632
Cystic	8	3.6	4	50.0	0	0	4	50.0	
Mixed	106	47.1	24	22.6	44	41.5	38	35.9	
Tall Positive	115	51.1	37	32.2	37	32.2	41	35.6	0.090
configuration Negative	110	48.9	39	35.5	36	32.7	35	31.8	
Ecogenity Isoechoic	89	39.7	27	30.3	30	33.7	32	36.0	0.078
Hyperechoic	11	4.9	4	36.4	4	36.4	3	27.2	
Hypoechoic	64	28.6	18	28.1	26	40.6	20	31.3	
Mixed	39	17.3	11	28.2	13	33.3	15	38.5	
Unknown	22	9.7	6	27.3	9	40.9	7	31.8	
Calcification None	169	75.1	61	36.1	58	34.3	50	29.6	0.394
Rough	32	14.2	9	28.1	11	34.4	12	37.5	
Micro	24	10.7	8	33.3	9	37.5	7	29.2	
Vascularity None	188	83.6	64	30.03	56	29.8	68	36.2	0.705
Peripheral	18	8.0	7	8.9	5	27.8	6	33.3	
Intranodular	19	8.4	6	31.6	5	26.3	8	42.1	
Boundary Regular	192	85.4	69	35.9	77	40.1	46	24.0	0.340
Irregular	33	14.6	9	27.3	11	33.3	13	33.4	
Halo sign Negative	223	99.1	82	36.8	72	32.3	69	30.9	0.930
Positive	2	0.9	1	50.0	0	0	1	50.0	
Total	225	100	172	76.4	27	12.0	26	11.6	

USG: Ultrasonographic Vit D: vitamin D

**Table 2. Ultrasonographic images and Vit D levels**

		Vit D						Total	
		Deficient		Insufficient		Normal		N	%
		N	%	N	%	N	%		
Ultrasonography	Suspicious	90	52.3	17	63.0	20	76.9	127	56.4
	56.4%								
	Benign	82	47.7	10	37.0	6	23.1	98	43.6
	34.6%								
Total		172	100	27	100	26	100	225	100.0

Vit D: Vitamin D

When the patients in follicular neoplasm or suspicious for follicular neoplasm and suspicious for malignancy groups were included in the malignant group, the

malignancy rates became 3.5 %. Malignancy rates were 3.2 % (6/189) in women and 5.6 % (2/36) in men. When we evaluated FNAB and Vit D levels, there was not difference

between FNAB and vitamin D levels.

Considering the histopathological surgery results of the patients, it was seen that 25 patients (11.1%) out of 225 were operated. When we examined the histopathological surgery results we found that 10 of them (10/225) (4.44%) were found malignant and 15 of them (15/225) (6.66%) were found benign. All of the nodules diagnosed as histopathologically malign were papillary carcinomas. When we evaluated surgical histopathology results and Vit D levels we did not find any difference in terms of malignancy (Table3).

When we revised FNAB results of patients participating in the study and their histopathological surgery results (Table 4), we observed that 1 case whose FNAB results were inconclusive, were malignant. According to Bethesda classification a case with benign result and a case with atypia of undetermined significance were diagnosed as papillary carcinomas. Seven cases whose histopathological result was malignant were also diagnosed as malign with FNAB. Fifteen FNAB results detected as benign, were found to be benign after surgery. Seven malignant cases according to FNAB results were operated but one case chose to be treated in another center. We found that 3 cases with benign FNAB results were recommended surgery for their sizes of the nodules.

### DISCUSSION

Skin exposure and dietary intake are the two sources of Vit D. Its metabolic activity depends on activation through hydroxylation of the 25 followed by the 1 position of this molecule by cytochromes P450s, the final product is active 1,25 (OH)2D3. The action of Vit D occurs through its binding to Vit D receptor (VDR) in the nucleus. Then VDR forms a heterodimer with retinoid-X receptors and binds Vit D response elements (VDRE) on chromatin resulting in the regulation of the expression of some target cells. Binding of VDRE with VDR affects gene transcription. Besides being involved in mineral metabolism VDR regulates some metabolic processes, like immune response and cancer signaling.

Thyroid cancer (TC) is the most common endocrine malignancy worldwide. Besides risk factors such as exposure to ionizing radiation, chemical genotoxins and obesity, lack of protective factors, like Vit D deficiency have been implicated in TC increased incidence (23-25).

Low levels of Vit D are measured all over the world, and its determined rate is 59.4-65.0% . In almost all studies, with normal Turkish individuals, Vit D levels were found to be below normal limits (28-29). The season when the study was performed, genetic variations, our clothing style, limited intake of food high in Vit D, lack of outdoor

**Table 3. Surgical histopathological and Vit D results**

		Vit D							
		Deficient		Insufficient		Normal		Total	
		N	%	N	%	N	%	N	%
Histopathological results	Benign	8	53.3	4	26.7	3	20.0	15	60.0
	Malign	6	60.0	3	30.0	1	10.0	10	40.0
Total		14	100.0	7	100.0	4	100.0	25	100.0

**Table 4. Comparison between FNAB and histopathological surgery results**

		Histopathological surgery results					
		Malign		Benign		Total	
		N	%	N	%	N	%
FNAB	Non-diagnostic	1	10.0	1	6.7	2	8.0
	Benign	1	10.0	14	93.3	15	60.0
	Atypia of undetermined significance	1	10.0	0	0	1	4.0
	Follicular neoplasm or suspicious for follicular neoplasm	0	0	0	0	0	0
	Suspicious for malignancy/ malignant	7	70.0	0	0	7	28.0
Total		10	100.0	15	100.0	25	100.0

FNAB: Fine needle aspiration biopsy

physical activity due to the season must be considered as the reason of hypovitaminosis D in our country. In another study of ours we found 14.3 ng/mL Vit D levels in normal individuals (30). In our study the average level of Vit D was  $16.1 \pm 7.6$ . In Hekimsoy's study the mean serum 25(OH)D concentration was  $16.9 \pm 13.09$  ng/mL, with 74.9% of the subjects having 25(OH)D deficiency (<20 ng/mL), 13.8% having insufficiency (20-29.99 ng/mL), and 11.3% of the subjects having sufficient 25(OH)D ( $\geq 30$  ng/mL) levels. The present study determined similar results with 76.4% of the patients being deficient, 12.0% being insufficient and 11.6% being sufficient in terms of Vit D status. Levels were lower in women than in men ( $14.4 \pm 4.9$ . vs  $16.2 \pm 8.2$  ng/mL, respectively). These results were consistent with Hekimsoy's study ( $15.25 \pm$  ng/mL vs  $20.70 \pm 15.50$  ng/mL, respectively) (31).

Studies have shown associations between Vit D deficiency and breast, colon and prostate cancers (20-22). However the relationship between Vit D levels and thyroid cancer is unknown. In experimental studies using cell lines or preclinical models to assess Vit D effect on thyroid cancer, overexpression (26-27) of Cyp24A1 mRNA, VDR and also CYP27B1 was shown (32-36). Antiproliferative effect of Vit D on thyroid cancer was also determined (37-39). In some clinical studies protective effect (40-47) and in some no effect of Vit D was found on thyroid cancer (48-52). In limited number of studies Vit D was determined to be an increasing risk for thyroid cancer (53-54).

Solar irradiation which is the primary source of Vit D, can be estimated by the latitude. Although our country is in a latitude that benefits from high solar irradiation, Vit D deficiency is highly prevalent in Turkey. Our clothing style, skin type, limited intake of food high in Vit D in lack of outdoor physical activity must be considered as the reason of hypovitaminosis D. Moreover, genetic determinants has effect on host intrinsic pathways such as polymorphic cytochromes P 450s responsible for the activation of Vit D, and can impact Vit D interaction vit VDR (32, 38). Downstream pathways in VDR are also subject to wide genetic variability among populations (55-56). These genetic variations were also shown to be critical determinants for the potential preventive properties of Vit D in TC. An inverse relationship was determined between TC incidence and latitude (41, 42). In a country like ours which has a low latitude but low Vit D levels we sought studies about genetic variations in our population, but we were not able to find such studies. In a study held with nonwestern immigrants searching the Vit D status in Europe, found that Vit D levels was low in the Turkish groups in Europe (57). Although this result may be explained by covering clothes, in the study group there were Turkish unveiled adult women. We can not show the genetic effects on these results.

By wondering if there is a relationship between Vit D and TC in Turkey, we not only sought a correlation with surgery results of thyroid nodules but also the results of USG and FNAB of the nodules and Vit D levels.

We classified our Vit D levels as deficient, insufficient and normal. When Vit D levels and characteristics of the nodules were evaluated, we did not find significant difference in Vit D levels of the patients and the characteristics of the nodules. After we listed our nodules having one of those features, which were solidity, tall configuration, markedly hypoechoic, microcalcifications, intranodular vascularity, irregular margin, no halo as suspicious, no relationship between Vit D levels and malign-benign USG features was found. Then we classified our FNAB results according to Bethesda class identifications. There was also no relationship between Vit D levels and malign-benign FNAB results. We determined the same result with Vit D levels and histopathological surgery results.

Our results showed that there were no relationship between Vitamin D and USG characteristics, FNAB and also histopathological surgery results. However, when we examined our results retrospectively we found that a very small number of cases who were found malignant with surgery were found benign by biopsy. These cases were in suspicious category ultrasonographically, and also above 2.5 cm in size. All of those 3 cases had Vit D levels below 20 IU/ml. We think that more extensive studies are needed to assess the association between malignancy and large thyroid nodules associated with vitamin D deficiencies. There are several limitations for our study. Its timing which is between October 2012–October 2014 limits this study's effectiveness. Measurement of Vitamin D levels of patients are affected by the season and it caused a heterogenous vitamin D measurement at our study. Patients who participated in our study are also has a limited number and extensive studies are needed.

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