Serum 25-hydroxyvitamin D level is unreliable as a risk factor and prognostic marker in papillary thyroid cancer

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Background: Low levels of vitamin D and altered local vitamin D metabolism have been associated with the prevalence and aggressiveness of several cancers. However, the effect of vitamin D on papillary thyroid cancer (PTC) is controversial. This study aimed to evaluate the impact of preoperative serum vitamin D levels and local vitamin D metabolism on the clinicopathologic characteristics and prognosis of PTC.

Methods: In total, 1,578 patients with PTC and 128 patients with benign thyroid diseases were included. Clinical and pathologic data were analyzed to evaluate the role of vitamin D as a risk factor and prognostic marker in PTC. Moreover, a tissue microarray was used to investigate the role of local vitamin D metabolism in PTC progression.

Results: Participants with PTC were younger compared to those with benign disease. No significant differences in 25-hydroxy vitamin D [25(OH)D] levels were observed between benign and malignant cases. Among patients with PTC, analyses of prognostic features revealed that decreased 25(OH)D levels were not overtly associated with poor prognosis in PTC. Additionally, local vitamin D metabolism was not associated with the aggressiveness of PTC.

Conclusions: Serum 25(OH)D determination may not contribute to risk assessment workup of thyroid nodules. Moreover, decreased preoperative serum vitamin D and local vitamin D metabolism were not associated with poor prognosis of PTC.

Keywords: 25-hydoxy vitamin D; papillary thyroid cancer (PTC); vitamin D metabolism; prognosis

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Introduction

Thyroid cancer is the most common endocrine malignancy and ranks fifth in cancer incidence in female malignant tumors in the United States (1). In China, thyroid cancer has become one of the most common malignant tumors in recent years and has been reported to be the most common malignant tumor in women younger than 30 years (2). Recently, the overall incidence of thyroid cancer has become higher in China than in the world (3). Most thyroid cancers derived from the follicular thyroid cell are well-differentiated thyroid cancers (DTCs), including the papillary (PTC) or follicular subtype (FTC), and have excellent survival rates (>90% 10-year survival) after standard treatment, consisting of thyroidectomy in most cases followed by treatment with radioactive iodine (4). However, persistent and recurrent PTC remains a therapeutic challenge, especially in radioactive refractory cases (5).

Vitamin D is a fat soluble vitamin and is critical to regulate calcium and phosphate metabolism. It is also involved in inflammatory reactions, angiogenesis, cell apoptosis, cell differentiation, and cell proliferation (6). Previous epidemiological studies have found low levels of 25-hydroxy vitamin D [25(OH)D] to be associated with a high risk of cancer, including colon, breast, and prostate cancer (7-9). In addition, 1,25(OH)D, the active form of vitamin D, has been shown to reduce both the incidence and the volume of tumors in animals (10,11). One study reported that vitamin D deficiency has an inverse relation with the incidence of DTC (12). Also, Kim *et al.* found that low serum vitamin D is associated with poor clinicopathologic characteristics in female PTC patients (13). However, the results of observational studies linking 25(OH)D levels with cancer incidence have been controversial. In fact, some reports have shown an increased risk of pancreatic cancer in patients with elevated levels of 25(OH)D (14-16).

1,25(OH)D exerts its biological activity through binding to the vitamin D receptor (VDR), a nuclear receptor. Previous studies reported that VDR polymorphisms and polymorphisms of the genes encoding for proteins influencing circulating 25(OH)D concentrations have been associated with cancer incidence (17,18). The metabolism of 1,25(OH)D is regulated by a complex process, involving the vitamin D-activating enzyme CYP27B1, responsible for the final hydroxylation step from 25(OH)D to 1,25(OH)D, and the 1,25(OH)D inactivated enzyme, CYP24A1. Thus, altered vitamin D metabolism may play a role in the progression of thyroid cancer (19).

The primary purpose of this study was to evaluate the associations of preoperative serum 25(OH)D on the various clinicopathologic features in patients with PTC. In total, 1,578 patients with PTC and 128 patients with benign thyroid disease were involved in. In addition, we also analyzed the local vitamin D metabolism to better revealed the role of vitamin D in PTC.

We present the following article in accordance with the REMARK reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-10/rc).

Methods

Study population

From May 2017 to June 2018, patients undergone thyroidectomy at Ruijin Hospital were retrospectively enrolled. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This research was approved by the Ethics Committee and the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (No. Ruijin LL-14-2006). Informed consent was taken from all the patients. A blood sample for evaluation was obtained before thyroid surgery. Patients who had any prior cancer history, daily vitamin D supplement, or disease that would affect serum vitamin D levels were excluded from the study. In total, 1,578 patients with PTC and 128 patients with benign thyroid disease met the inclusion criteria for analysis in this study. Medical records of these patients were retrospectively reviewed to obtain the following data: age at diagnosis, sex, anthropometric traits, preoperative parathyroid hormone (PTH), ionized calcium, ionized phosphorus, 25(OH)D, thyroid function, and clinicopathologic characteristics. 25(OH)D levels were detected by high performance liquid chromatography (HPLC). Vitamin D deficiency is defined as a 25(OH)D below 20 ng/mL, and vitamin D insufficiency as a 25(OH)D of 21–29 ng/mL based on The Endocrine Society Clinical Practice Guideline (20).

Immunohistochemistry

Immunohistochemistry was performed as previously described (21). Briefly, paraffin-embedded tumor specimens were subjected to a heat pretreatment at 60 ℃ for 1 hour, dewaxed in xylene, rehydrated in a series of ethanol, and

Table 1 Clinical characteristics of patients with benign thyroid nodules and papillary thyroid cancer

Characteristics	Before matched			After matched		
	Thyroid benign disease (n=128)	Papillary thyroid cancer (n=1,578)	P value	Thyroid benign disease (n=128)	Papillary thyroid cancer (n=127)	P value
Age (years)	52.70±13.48	43.97±12.51	<0.001	52.70±13.48	52.07±13.43	0.708
female/male (%)	101/27 (21.1)	1218/360 (22.8)	0.655	101/27 (78.9/21.1)	95/32 (74.8/25.2)	0.437
BMI (kg/m^2)	23.07±3.23	23.08±3.35	0.975	23.07±3.23	23.20±3.17	0.754
Preoperative TSH (μIU/mL)	1.76±1.24	1.94±1.48	0.116	1.76±1.24	1.87±1.06	0.433
Preoperative PTH (pg/mL)	60.10±32.26	57.62±22.88	0.396	60.10±32.26	62.94±22.61	0.417
Preoperative FT3 (pg/mL)	4.33±0.51	4.40±0.65	0.238	4.33±0.51	4.40±0.49	0.280
Preoperative FT4 (pmol/L)	13.08±1.79	13.31±1.59	0.151	13.08±1.79	13.06±1.33	0.929
25 hydroxy vitamin D (ng/mL) (mean)	18.58±7.67	17.49±6.74	0.123	18.58±7.67	18.38±7.42	0.836
25 hydroxy vitamin D (ng/mL) (median)	18.06 (5.71–39.90)	16.84 (4.57–52.32)		18.06 (5.71–39.90)	17.70 (4.57–45.43)	
Preoperative ionized Ca (mg/dL)	2.27±0.11	2.30±0.11	0.005	2.27±0.11	2.30±0.10	0.017
Preoperative P (mmol/L)	1.15±0.15	1.16±0.17	0.527	1.15±0.15	1.14±0.0.15	0.655

BMI, body mass index; TSH, thyroid stimulating hormone; PTH, parathyroid hormone; FT3, free triiodothyronine; FT4, free thyroxine.

treated with 0.01 mol/L citrate buffer (pH 6.0) for antigen retrieval. After inhibition of endogenous peroxidase activity for 30 minutes with methanol containing 0.3% H_2O_2, the sections were stained with anti-VDR (1:200, Abcam), anti-CYP27B1 (1:200, Abcam), or anti-CYP24A1 antibody at 4 ℃ overnight, followed by incubation by horseradish peroxidase (HRP)-labeled secondary antibody. The staining intensity was scored from 0 to 3 (0 = negative, 1 = mild staining, 2 = moderate staining, and 3 = strong staining) for every cell. An H-score is given using the following formula: [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]. The final score was within 0-300 to quantify the protein expression.

Statistical analyses

Statistical analyses were performed using IBM SPSS for Windows v.21.0 (IBM Corp., Armonk, NY, USA). A chi-square test was used to compared categorical variables, while Student's *t* test was used for continuous variables. Pearson's correlation method was used to determine the correlation between serum vitamin D and other parameters. Logistic regression analyses were used to evaluate the effect of vitamin D on the aggressiveness of thyroid cancer. A P value of <0.05 was considered to be statistically significant.

Results

Benign thyroid nodular diseases vs. thyroid carcinomas

In total, 1,578 patients with PTC and 128 patients with benign thyroid disease met the inclusion criteria for analysis in this study. Patients with PTC were younger than those with benign thyroid disease (mean age 43.97±12.51 *vs.* 52.70±13.48 years; P<0.001; *Table 1*). 25(OH)D deficiency (<20 ng/mL) was present in 63.3% (81/128) of those with benign nodules and 69.8% (1,102/1,578) of those with PTC (P=0.122). There was also no significant difference in serum 25(OH)D between patients with benign *vs.* malignant thyroid disease (mean: 18.58±7.67 *vs.* 17.49±6.74; P=0.123). We then used case–control analysis according to age, sex, body mass index (BMI), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), and parathyroid hormone (PTH). A total of 127 malignant and 128 benign cases were enrolled. We also found no statistically significant difference in serum 25(OH)D between the 2 groups (*Table 1*).

Clinicopathologic characteristics according to vitamin D level

Serum 25(OH)D was significantly lower in patients who

Table 2 Relationship between 25-hydroxy vitamin D levels and the clinicopathologic characteristics of papillary thyroid cancer

Variables	No. of patients (%)	25 (OH) vitamin D (ng/mL)	P value
Age			<0.001
<45 years	846 (53.61)	16.49±6.05	
≥45 years	732 (46.39)	18.65±7.29	
Sex			<0.001
Male	360 (22.81)	19.88±7.16	
Female	1218 (77.19)	16.79±6.44	
Tumor size			0.622
≤1 cm	1117 (70.79)	17.55±6.57	
>1 cm	461 (29.21)	17.36±7.14	
Multifocality			0.070
Negative	462 (29.28)	17.97±6.95	
Positive	1116 (70.72)	17.30±6.64	
Bilateral			0.287
Negative	1287 (81.56)	17.41±6.68	
Positive	291 (18.44)	17.87±6.97	
Central lymph node metastasis			0.276
Negative	875 (55.45)	17.66±6.73	
Positive	703 (44.55)	17.29±6.75	
Lateral lymph node metastasis			0.766
Negative	1435 (90.94)	17.51±6.77	
Positive	143 (9.06)	17.33±6.39	
Stage			<0.001
I/II	1308 (82.89)	17.15±6.54	
III/IV	270 (17.11)	19.14±7.41	
Hashimoto's thyroiditis			0.003
Negative	1467 (92.97)	17.63±6.79	
Positive	111 (7.03)	15.64±5.71	

were <45 years old than in those who were ≥45 years old (16.49±6.05 *vs.* 18.65±7.29; P<0.001; *Table 2*). Serum 25(OH) vitamin D (same as before) levels were also significantly decreased in patients with Hashimoto's thyroiditis (15.64±5.71 *vs.* 17.63±6.79; P=0.003). However, reduced serum 25(OH) vitamin D showed no statistically significant association with tumor size, central lymph node metastasis (LNM), lateral LNM, or positive results for multifocality or bilateral thyroid carcinoma. Unexpectedly, serum 25(OH) vitamin D levels were higher in patients with stage III/IV PTC (19.14±7.41 *vs.* 17.15±6.54, P<0.001).

Since vitamin D insufficiency or deficiency (<30 ng/mL) was observed in most PTC patients (95.5%), they were divided into 4 groups according to serum vitamin D levels. Baseline characteristics were compared between each quartile group, but no significant associations were found with BMI or TSH (*Table 3*). In the fourth quartile group, the age at diagnosis of thyroid cancer was significantly older,

Table 3 Clinicopathological characteristics according to quartiles of serum 25-hydroxyvitamin D

Variables	Total(N=1,578)	Quartile 1 (<12.46) (N=395)	Quartile 2 (12.47–16.84) (N=395)	Quartile 3 (16.86–21.06) (N=394)	Quartile 4 (21.06–52.32) (N=395)	P value
25(OH)D	17.49±6.74	9.90±1.83	14.79±1.34	18.86±1.22	26.44±5.29	<0.001
Age (years)	43.97±12.51	41.46±12.09	42.63±12.53	44.46±12.17	47.34±12.49	<0.001
Sex, male	360 (22.8%)	50 (12.7%)	82 (20.8%)	96 (24.4%)	132 (33.5%)	<0.001
BMI (kg/m^2)	23.08±3.35	23.04±3.58	23.10±3.38	23.03±3.29	23.16±3.12	0.948
Tumor size (cm)	0.93±0.79	1.00±0.95	0.97±0.80	0. 87±0.70	0.88 ±0.69	0.069
Multifocality	462 (29.3%)	106 (26.8%)	116 (29.4%)	108 (27.4%)	132 (33.5%)	0.158
Central LNM	703 (44.6%)	187 (47.3%)	183 (46.3%)	172 (43.7%)	161 (40.9%)	0.256
Lateral LNM	143 (9.1%)	37 (9.4%)	37 (9.4%)	35 (8.9%)	34 (8.6%)	0.979
Stage III/IV	270 (17.1%)	53 (13.4%)	57 (14.4%)	70 (17.8%)	90 (22.8%)	0.002
Bilateral	291 (18.4%)	72 (18.2%)	67 (17.0%)	73 (18.5%)	79 (20.1%)	0.737
Hashimoto's thyroiditis	111 (7.0%)	39 (9.9%)	29 (7.3%)	30 (7.6%)	13 (3.3%)	0.004
Preoperative TSH (μIU/mL)	57.62±22.88	62.85±25.74	57.44±22.94	56.72±20.91	53.48±20.66	0.102
Preoperative PTH (pg/mL)	57.62±22.88	62.85±25.74	57.44±22.94	56.72±20.91	53.48±20.66	<0.001
Ionized Ca (mg/dL)	2.30±0.11	2.28±0.11	2.29±0.11	2.31±0.10	2.31±0.10	<0.001
Preoperative P (mmol/L)	1.16±0.17	1.16±0.15	1.15±0.16	1.16±0.16	1.15±0.21	0.774
Preoperative FT3 (pg/mL)	4.40±0.65	4.35±0.51	4.35±0.46	4.42±0.47	4.49±0.98	0.005
Preoperative FT4 (pmol/L)	13.31±1.59	13.32±1.75	13.34±1.65	13.28±1.50	13.31±1.47	0.962

BMI, body mass index; LNM, lymph node metastasis; TSH, thyroid stimulating hormone; PTH, parathyroid hormone; FT3, free triiodothyronine; FT4, free thyroxine.

and there were significantly more males. Meanwhile, PTH decreased (P<0.001), ionized Ca (P<0.001) and preoperative FT3 (P=0.005) increased from the first quartile to the fourth quartile. A right-skewed distribution was observed in patients with stage III/IV (13.4%, 14.4%, 17.8%, 22.8%; P=0.002), and a left-skewed distribution was noticeable in patients with Hashimoto's thyroiditis (9.9%, 7.3%, 7.6%, 3.3%; P=0.004). Positive correlations were shown between age, Ca, FT3, and serum vitamin D levels. In contrast, negative correlations were demonstrated between serum PTH, TSH, and adjusted vitamin D levels (*Table 4*).

Effect of serum vitamin D on the aggressiveness of PTC

Table 5 shows the results of a univariate logistic regression with unadjusted odds ratio (ORs) for which the fourth quartile was established as a reference (unadjusted OR for all parameters = 1). Compared to the fourth quartile, the first quartile showed an unadjusted OR of 0.73 for multifocality [95% confidence interval (CI): 0.54–0.99; P=0.042] and 0.52 for stage III/IV (95% CI: 0.36–0.76; P=0.001). Only the risk of stage III/IV disease was significant (OR 0.57, 95% CI: 0.40–0.82; P=0.003) in the second quartile compared to the fourth quartile.

Multivariate logistic regression analysis was conducted to examine the interaction between poor clinicopathologic features and age, sex, TSH, PTH, Hashimoto's thyroiditis, and preoperative ionized calcium on serum 25(OH) vitamin D (*Table 6*). Tumor size >1 cm, advanced cancer stages (III or IV), central LNM, lateral LNM, and bilateral and multifocal thyroid carcinoma did not significantly differ between the serum vitamin D quartiles.

Effect of serum vitamin D metabolism on the aggressiveness of PTC

In order to investigate the role of VDR, CYP27B1, and CYP24A1 in PTC progression, we first evaluated

Table 4 Correlations of serum 25-hydroxyvitamin D levels with sex, age, body mass index, and thyroid function tests

Variables	N	Pearson's correlation	P value
Age	1,578	0.174	<0.001
BMI	1,578	0.010	0.702
PTH	1,578	–0.141	<0.001
Ca	1,578	0.123	<0.001
P	1,578	–0.017	0.501
TSH	1,578	–0.058	0.022
FT3	1,578	0.086	0.001
FT4	1,578	–0.006	0.816

BMI, body mass index; TSH, thyroid stimulating hormone; PTH, parathyroid hormone; FT3, free triiodothyronine; FT4, free thyroxine.

Table 5 Odds ratios and confidence intervals for various prognostic factors and thyroid cancer stages in relation to 25-hydroxyvitamin D quartiles

Variables	Quartile 1		Quartile 2		Quartile 3		Quartile 4
	OR [95% CI]	P value	OR [95% CI]	P value	OR [95% CI]	P value	
Tumor >1 cm	1.21 [0.89–1.65]	0.220	1.24 [0.91–1.69]	0.168	0.94 [0.68–1.29]	0.688	1 (reference)
Multifocality	0.73 [0.54–0.99]	0.042	0.83 [0.61–1.12]	0.211	0.75 [0.55–1.02]	0.064	1 (reference)
Bilateral	0.89 [0.62–1.27]	0.515	0.81 [0.57–1.17]	0.264	0.91 [0.64–1.29]	0.588	1 (reference)
Central LNM	1.30 [0.98–1.72]	0.067	1.25 [0.94–1.66]	0.122	1.12 [0.85–1.49]	0.428	1 (reference)
Lateral LNM	1.09 [0.67–1.78]	0.717	1.09 [0.68–1.78]	0.717	1.03 [0.63–1.69]	0.900	1 (reference)
Stage III/IV	0.52 [0.36–0.76]	0.001	0.57 [0.40–0.82]	0.003	0.73 [0.52–1.04]	0.077	1 (reference)

LNM, lymph node metastasis; OR, odds ratio; CI, confidence interval.

the messenger RNA (mRNA) expression level of VDR, CYP27B1, and CYP24A1 in 2 Oncomine databases (*Figure 1A-1C*). The results showed that CYP27B1 expression was significantly lower in PTC tissue than that in normal tissue (P<0.05). However, the levels of VDR and CYP24A1 did not significantly differ between PTC and normal tissues. Moreover, Kaplan-Meier survival curves showed that the mRNA levels of VDR, CYP27B1, and CYP24A1 did not significantly affect the overall survival of PTC patients in a cohort from The Cancer Genome Atlas (TCGA; P>0.05; *Figure 1D-1F*). Furthermore, 60 PTC tissues were used to detect the correlation between the protein expressions of VDR, CYP27B1, and CYP24A1 and clinicopathologic features (*Figure 1G-1I*). Statistical analyses revealed that there was no significant difference in the levels of VDR, CYP27B1, or CYP24A1 for several poor clinicopathologic features (*Table 7*).

Discussion

Previous research has found that serum 25-(OH)D levels are negatively correlated with the risk of an array of tumor types (22). Several studies have also shown that vitamin D might play a role in the development, progression, and treatment of cancers (23,24). Thyroid cancer is the most common endocrine tumor in the world, and whether the lack of vitamin D increases or not thyroid cancer risk, has become a topic of intense research interest. The role of vitamin D in thyroid cancer is still controversial. Numerous studies have shown that vitamin D deficiency is associated with an increased risk for thyroid cancer (25,26). A retrospective cohort among 212 DTC patients also showed that vitamin D deficiency (<15 ng/mL) may increase the risk of developing DTC (12). Previous studies have found that serum 1,25(OH)D in patients with thyroid cancer is low, and the higher the degree of malignancy, the lower the

Table 6 Logistic regression analysis of the effect of 25-hydroxyvitamin D on the aggressiveness of thyroid cancer

Variables	Model 1			Model 2		
	OR	95% CI	P value	OR	95% CI	P value
Tumor >1 cm						
Quartile 1	1.22	0.89–1.68	0.215	1.31	0.94–1,81	0.107
Quartile 2	1.24	0.91–1.69	0.178	1.28	0.94–1.76	0.120
Quartile 3	0.94	0.68–1.29	0.691	0.95	0.69–1.31	0.761
Quartile 4	1	Ref		1	Ref	
Multifocality						
Quartile 1	0.762	0.56–1.04	0.090	0.73	0.53–1.01	0.057
Quartile 2	0.86	0.63–1.16	0.320	0.84	0.61–1.14	0.256
Quartile 3	0.77	0.57–1.04	0.910	0.75	0.55–1.02	0.066
Quartile 4	1	Ref			1	Ref
Bilateral						
Quartile 1	0.96	0.66–1.38	0.808	0.91	0.63–1.33	0.63
Quartile 2	0.86	0.60–1.24	0.424	0.84	0.58–1.21	0.348
Quartile 3	0.94	0.66–1.34	0.736	0.91	0.63–1.30	0.602
Quartile 4	1	Ref		1	Ref	
Central LNM						
Quartile 1	1.33	0.99–1.79	0.059	1.32	0.98–1.79	0.072
Quartile 2	1.23	0.92–1.65	0.165	1.23	0.92–1.65	0.168
Quartile 3	1.12	0.84–1.50	0.46	1.11	0.83–1.49	0.486
Quartile 4	1	Ref		1	Ref	
Lateral LNM						
Quartile 1	1.17	0.71–1.94	0.545	1.07	0.64–1.80	0.790
Quartile 2	1.12	0.68–1.84	0.657	1.06	0.64–1.76	0.812
Quartile 3	1.06	0.64–1.74	0.831	1.03	0.62–1.70	0.914
Quartile 4	1	Ref		1	Ref	
Stage III/IV						
Quartile 1	0.98	0.64–1.51	0.93	0.97	0.62–1.51	0.876
Quartile 2	0.91	0.60–1.39	0.668	0.91	0.60–1.39	0.666
Quartile 3	0.97	0.65–1.44	0.864	0.95	0.64–1.43	0.819
Quartile 4	1	Ref				

Model 1 was adjusted for age (<45 and ≥45 years) and sex; model 2 was adjusted for the variables in model 1 plus TSH, PTH, FT3, Hashimoto's thyroiditis, and preoperative ionized calcium. CI, confidence interval; OR, odds ratio; LNM, lymph node metastasis.

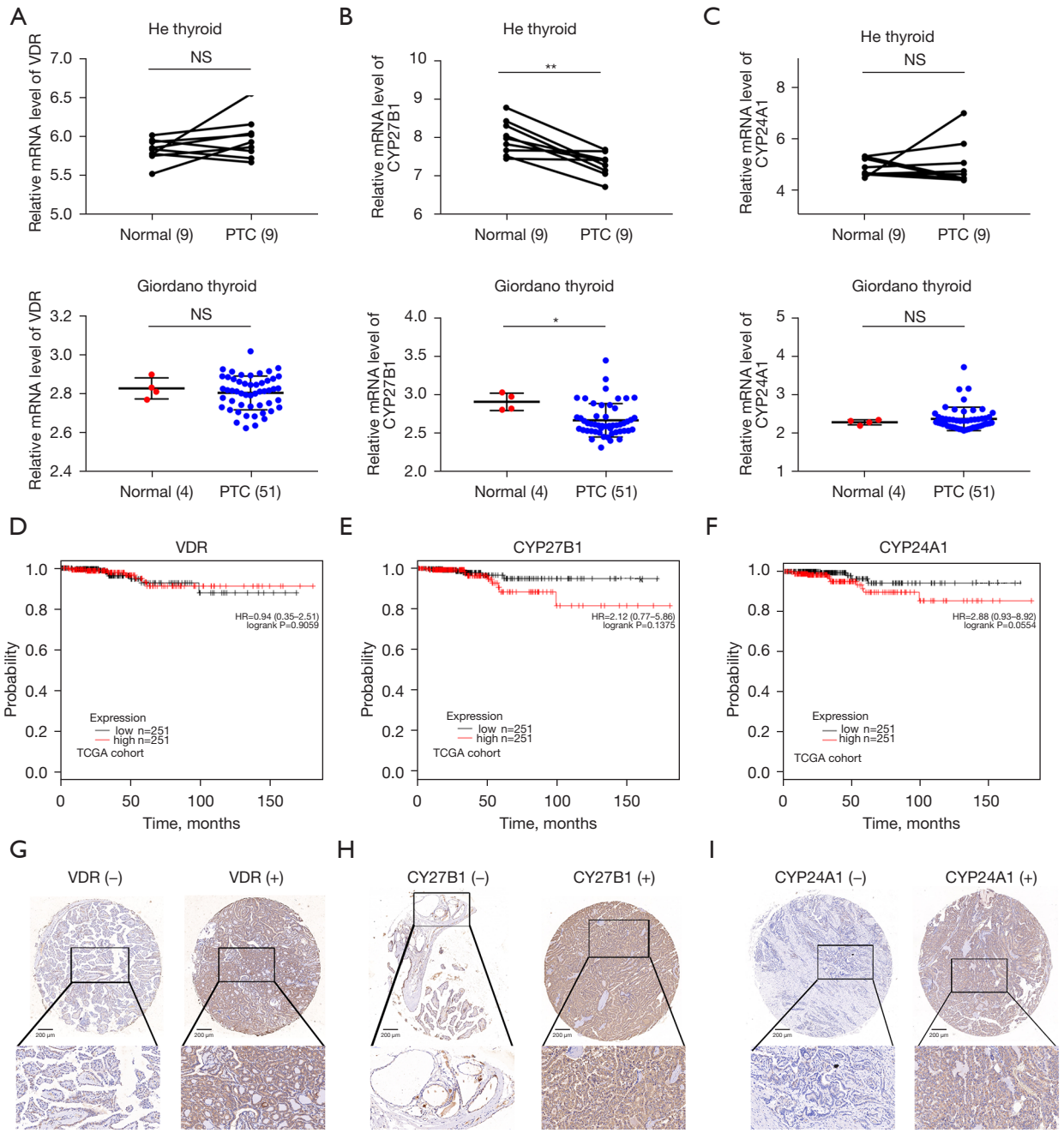

Figure 1 The levels of VDR, CYP27B1 and CYP24A1 are not associated with the aggressiveness of PTC. (A-C) The relative mRNA expressions of VDR, CYP27B1, and CYP24A1 in 2 Oncomine data sets: He thyroid (normal =9, PTC =9) and Giordano thyroid (normal =4, PTC =51). (D-F) Kaplan-Meier analysis of overall survival in 60 patients with PTC (TCGA database). (G-I) VDR, CYP27B1, and CYP24A1 expression levels in tumor tissues were evaluated by immunohistochemical staining with tissue microarray (5×, 40×). *, P<0.05; **, P<0.01; NS, P>0.05. VDR, vitamin D receptor; PTC, papillary thyroid cancer.

concentration of 1,25(OH)D (27). Another retrospective study that included 548 female PTC patients, reported that the lower the vitamin D levels are (<18.57 ng/mL), the larger the thyroid tumors and the greater the possibility of metastasis (13). A single clinical case has also been reported that the size of the thyroid cancer decreases after treatment

Table 7 Relationship Between VDR, CYP27B1 and CYD24A1 levels and the clinicopathologic characteristics of papillary thyroid cancer

Variables	No. of patients (%)	VDR		CYP27B1		CYD24A1	
		H-score	P value	H-score	P value	H-score	P value
Sex			0.491		0.050		0.394
Male	12 (20.0)	138.31±62.01		172.23±19.08		56.78±47.96	
Female	48 (80.0)	127.71±43.18		155.52±27.16		43.83±34.51	
Age			0.059		0.300		0.014
<45	34 (56.7)	139.84±49.36		162.24±18.40		56.24±40.85	
≥45	26 (43.3)	116.73±41.35		154.44±34.22		33.57±28.45	
Extrathyroidal extension							
Negative	48 (80.0)	133.44±48.89	0.238	158.99±28.10	0.938	48.55±38.37	0.380
Positive	12 (20.0)	115.38±37.60		158.32±19.59		37.85±33.81	
Multifocality					0.448		0.913
Negative	49 (81.7)	128.79±46.25	0.748	157.62±27.96		46.67±37.98	
Positive	11 (18.3)	134.45±52.98		164.38±18.47		45.30±36.88	
Tumor size			0.259		0.103		0.665
≤1	16 (26.7)	141.30±41.81		167.78±23.98		49.85±36.02	
>1	44 (73.3)	125.66±48.68		155.62±26.84		45.17±38.32	
T stage			0.106		0.371		0.246
1/2	52 (86.7)	133.69±46.11		160.07±27.18		48.63±38.18	
3/4	8 (13.3)	104.71±48.94		151.00±21.00		32.02±30.77	
N stage			0.148		0.763		0.397
0	30 (50.0)	120.98±49.80		157.82±31.16		42.28±37.60	
1	30 (50.0)	138.67±43.31		159.90±21.26		50.56±37.53	
M stage			NS		NS		NS
0	59 (98.3)	129.72±47.52		158.44±26.50		46.61±37.77	
1	1 (1.7)	136.04		183.64		35.01	

VDR, vitamin D receptor.

with vitamin D (28). Further, *in vitro* and animal studies have shown that vitamin D can inhibit cell proliferation, increase cell adhesion, and promote the differentiation of thyroid cancer cells (10,29-31).

However, the role of vitamin D in thyroid cancer remains controversial (32). In contrast, several studies did not find any association between serum 25(OH)D and thyroid cancer. Warakomski *et al.*, found no significant relationships between serum vitamin D and tumor size in PTC (33), while Jonklaas *et al.* reported that there was no association between preoperative serum 25(OH)D levels and thyroid cancer diagnosis, disease stage, or any other prognostic characteristics among 65 patients with thyroid cancer (34). Lizis-Kolus *et al.* also found no relationship between serum 25(OH)D levels and the disease stage of patients with PTC (35). Moreover, a study by Ahn *et al.* of 820 PTC patients found that preoperative serum 25(OH)D levels were not associated with prognosis or aggressiveness (14). In the present study, we included 1,578 PTC patients and 128 patients with benign thyroid diseases to further clarify

the role of vitamin D in the pathogenesis and progression of thyroid cancer.

We found that lower preoperative serum vitamin D levels were not associated with a higher aggressiveness of PTC. The above results were confirmed in patients with and without thyroiditis and in both male and female populations. However, no evidence for an association between the aggressiveness and vitamin D levels was found (data not shown). The results suggest that vitamin D levels might not significantly impact the biological behavior of PTC. In China, vitamin D insufficiency is prevalent in PTC patients. Therefore, patients in our study were divided into 4 groups according to preoperative serum vitamin D levels. The vitamin D levels in the first group were the lowest. Although larger tumor size and a greater proportion of LNM cases were found in the first group, more advanced stages (III/IV) were observed in the fourth group. These discrepancies might suggest that vitamin D is not a reliable indicator for the aggressiveness of thyroid cancer.

VDR level and local vitamin D metabolism have been proposed to influence the effect of vitamin D on thyroid cancer. A previous study showed that VDR expression was increased in PTC compared with normal thyroid tissue and was especially higher in areas of lymphocyte infiltration (36). In our study, we found the CYP27B1 level was lower in PTC than in normal tissues, but VDR and CYP24A1 did not significantly differ between PTC and normal tissues. Moreover, we found that the expression of VDR, CYP27B1, and CYP24A1 may not be associated with the aggressiveness of PTC or the overall survival of PTC patients. Therefore, the degree to which vitamin D and related proteins affect the development of thyroid cancer, if at all, remains controversial. According to our studies, vitamin D may do not actually affect PTC's malignant biological properties. However, some experimental researches find that vitamin D shows anti-tumor ability or enhances doxorubicin-induced apoptosis in PTC through Wnt/β-catenin or VDR/PTPN2/p-STAT3 signaling pathway (37,38). Vitamin D may be able to inhibit PTC in on a cellular level. If our study contain the long-term follow-up data, we would draw a more credible conclusions.

The present study has several limitations. First, we used a retrospective single-center design and included only Asian patients. Second, long-term follow-up data in this patient cohort are lacking. Third, the proportion of patients with sufficient vitamin D and the number of tissues for immunohistochemistry were small.

Despite these limitations, this study included a larger number of patients to investigate the clinicopathologic features and aggressiveness of PTC, providing more reliable evidence for revealing the role of vitamin D in PTC. However, further clinical trials including randomized controlled trials should be performed to more clearly determine the effects of vitamin D on PTC.

Conclusions

We conducted a retrospective single-center study of PTC patients with the aim of evaluating the associations of preoperative serum 25(OH)D and local vitamin D metabolism on the various clinicopathologic features in patients with PTC. No clear association between preoperative serum vitamin D levels or local vitamin D metabolism and PTC risk or aggressiveness of PTC was found. These results are in keeping with previous studies and suggest that Serum 25(OH)D determination may not contribute to risk assessment workup of thyroid nodules.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-10/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-10/coif). JJD received honoraria for lectures from Lilly, Faes, Menarini, MSD and Takeda, and received support for attending meetings and/or travel from Takeda, Menarini and Ipsen. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This research was approved by the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (No. Ruijin LL-14-2006). Informed consent was taken from all the patients.

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