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ORIGINAL ARTICLE

Study of Correlation between TSH Levels and VEGF Expression in Thyroid Lesions

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ABSTRACT
Vascular endothelial growth factor (VEGF) stimulates angiogenesis, increases vascular permeability and seems to correlate to aggressiveness of tumors. Increased expression of VEGF has been observed in thyroid carcinoma. Thyroid stimulating hormone (TSH) is the major thyroid hormone, but its relationship with VEGF has seldom been studied. To study the clinical and demographic profile of thyroid lesions (benign and malignant) and to explored the relationship between VEFG expression (using immunochemistry) and serum TSH level. This prospective, observational study includes 61 patients of thyroid lesions who underwent partial, hemi, subtotal or total thyroidectomy as the primary treatment from June 2014 and July 2016. Tissue specimens of thyroid lesions for immunohistochemistry study of VEGF expression were done. Serum TSH was done using Chemiluminiscence technique and correlated to VEGF expression. The mean age of patient was 36.26±11.53 years (range 20-50 years) with female preponderance. Swelling was the most common presenting symptom. Of 61 patients, 37 (60.65%) patients were benign and 24 (39.35%) were malignant thyroid lesions. The mean TSH level in benign group was 1.92±0.94 mIU/liter and malignant group was 2.73±1.74 mIU/liter which was statistically significant (p=0.023). VEGF expression was strongly positive (3+) in 26 (42.62%) patients and negative/equivocal (1+ & 2+) in 35 (57.38%) patients. In benign group, 10 (27.0%) patients were strongly positive for VEGF whereas in malignant group, 16 (66.7%) patients were strongly positive for VEGF showed significant association (p=0.002). On comparing TSH level of benign, malignant and total patients separately with VEGF expression, significant association were also observed (p<0.001, p=0.004 and p<0.001 respectively). VEGF was strongly expressed in malignant thyroid lesions which are having high serum concentration of serum TSH level. Serum TSH levels reveal a significant correlation with VEGF expression.
Key words: Thyroid lesion, Vascular endothelial growth factor, Thyroid stimulating hormone

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INTRODUCTION

Thyroid disorders are globally the most common endocrine disorder. India is no exception to this phenomenon around the world too. It is estimated that approximately 42 million Indians suffer from thyroid diseases [1]. Thyroid disorders differ from other diseases in terms of their ease of diagnosis, quality of medical treatment and the relative exposure that even a minor thyroid swelling provides to the treating doctor. There are various well-established malignancy predictors in thyroid nodules, including finding hard and fixed lesions, rapid progression of nodule, dysphagia, lymphadenopathy or associated hoarseness, although all of these are relatively uncommon in diagnosis [2]. Certain risk factors include age (<20 years or >70 years), male sex and previous history of exposure to irradiation.

TSH is the primary thyroid stimulating hormone and plays a significant role in the thyroid gland growth and functioning [3]. Various studies have shown that higher TSH concentrations in patients with thyroid nodules, also within the limit, are correlated with a subsequent diagnosis of thyroid cancer [4,5]. In addition, elevated serum TSH levels correlated with advanced stage thyroid cancer have been found [6]. Such results indicate that TSH may play a significant role in thyroid carcinoma formation and/or progression.

Angiogenesis is well known to play a major role in cancer formation, growth and metastasis and VEGF has been identified as a key angiogenesis mediator in the thyroid gland [7,8]. In human thyroid cancer, the upregulation of

VEGF is associated with malignancy and poor prognosis [9-10]. Several research demonstrated a strong association between expression of VEGF and in vitro concentration of serum TSH [11,12]. Nonetheless, very limited data are available on the association between expression of VEGF and concentration of serum TSH in human tissue, which has been the subject of this research.

MATERIAL AND METHODS

This is a prospective, observational hospital based study which was done after obtaining ethical approval from the Ethical Committee of Institute of Medical Sciences, Banaras Hindu University from June 2016 and July 2018. The study includes 61 patients of metastatic and non-metastatic thyroid cancer and benign thyroid lesions (Grave's thyroidits, Hashimoto's thyroiditis and Colloid goiter). Patients with presence of associated malignancies, patients on thyroxin or antithyrod medications, steroid intake and pregnancy were excluded from the study. A written informed consent was taken from each patient prior to evaluation.

A detailed history, clinical evaluation, routine blood investigations, thyroid function tests (T3, T4 and TSH), X-ray neck, ultrasound of thyroid and fine needle aspiration cytology (FNAC) were performed in all the patients. Before taking written informed consent, patients underwent operation (hemithyroidectomy or partial, subtotal and total thyroidectomy). The serum TSH was done using Chemiluminiscence technique in Endochrinology Lab of University hospital.

Tissue specimens of thyroid lesions for immunohistochemistry study of VEGF expression were done using antibodies in formalin fixed and paraffin embedded histological sections. Rabbit anti human polyclonal antibodies to both receptors obtained from purified immunoglobin fractions, diluted in PBS, pH 7.6, 1% BSA and .09% sodium azide. The secondary antibody was biotinylated rabbit anti-goat IgG. Antibody and detection kit was obtained from "BIOGENEX" through a proper agency. Tissue sections were cut into slices at approximately 0.5 cm and the specimen were kept in 10% buffered formalin for 18-24 hours for proper fixation, then the specimen were grossed by trained pathologist to obtain representative tissue sections which were processed routinely in the conventional way for embedding in paraffin wax. 4µm section were cut and placed on glass slide, one slide of each tissue was stained with Hematoxylin and Eosin (H & E). After H & E staining these sections were evaluated under light microscopy for histopathological details. Blocks of the viable tumor representative area were selected for immunohistochemistry staining with antibodies for the VEGF. A block of angiocarcinoma tissue provided by Biogenex served as positive control for VEGF staining.

After proper staining, VEGF stained slides were subjected to microscopic evaluation. Only invasive tumor cells were considered and the cytoplasmatic staining intensity was scored using a semi-quantitative scale. In statistical analysis, strong immunoreactivity (3+ or more) was taken as positive results whereas weak or absent staining (0 to 2+) was considered negative. Interpretation of immunohistochemical results was made without knowledge of clinical outcome and the status of other prognostic variables.

The statistical analysis was done using SPSS for Windows version 16.0 software (IBM Inc.). For categorical data Chi-square and Fischer's Exact test was used. For comparing two groups of mean Students 't' test was used. The critical value of 'p' indicating the probability of significant difference was taken as <0.05 for comparison.

RESULTS

The mean age of patients was 36.26±11.53 years (range 20-50 years) with female preponderance (n=50). Swelling was the most common presenting symptom. Euthyroid was the most common sign present in 54 (88.52%) patients followed by hypoyhyroidism in 6 (9.83%) and hyperthyroidism only 1 (1.63%) case. Solitary thyroid nodules were present in 30 (49.18%) patients while multi-nodular goiter in 61 (50.82%). Of 61 patients, 37 (60.65%) patients were benign and 24 (39.35%) were malignant thyroid lesions on histopathology. In benign group, multi-nodular goiter was present in majority of patients (n=12) followed by follicular adenoma (n=10), colloid goiter (n=6), adenomatous goiter (n=5), thyroiditis (n=3) and hurthle cell adenoma (n=1). In malignant group, majority of patients were papillary carcinoma (n=17) followed by follicular carcinoma (n=5) and medullary carcinoma (n=2). In benign group, solitary thyroid nodules were present in 12 (32.4%) patients and 25 (67.6%) patients were multi-nodular goiter while in malignant group, solitary thyroid nodules were present in 18 (75.0%) patients and multi-nodular in 6 (25.0%) patients which showed significant association ($p<0.001$). The relationship between demographic, clinical, laboratory and radiological characteristics of thyroid lesions in both benign and malignant is shown in Table 1.

The mean hemoglobin, T3, T4 and serum calcium level were comparable in both benign and malignant group ($p=0.380$, $p=0.708$, 0.877 and 0.145 respectively). The mean TSH level was significantly raised in

malignant group (2.73±1.74 mIU/liter) as compared to 1.92±0.94 mIU/liter in benign group (p=0.023) (Table 1). On ultrasonography, 32 (86.5%) patients were probably benign and 5 (13.5%) patients were suspiciously malignant in benign group whereas, in malignant group, 23 (95.8%) patients were suspiciously malignant and only 1 case was probably benign showed significant association (p<0.001) (Table 1).

Table 1: Relationship between demographic, clinical, laboratory and radiological characteristics of thyroid lesions

	Benign (n=37)	**Malignant (n=24)**	**p-value**
	No. (%)	**No. (%)**	
Age group (years)			
30-40	6 (16.2%)	13 (54.2%)	**0.004**
41-50	23 (62.2%)	10 (41.7%)	
>50	8 (21.6%)	1 (4.2%)	
Mean±SD	47.27±6.35	41.42±6.21	**0.001**
Gender			
Male	10 (27.0%)	1 (4.2%)	**0.023**
Female	27 (73.0%)	23 (95.8%)	
Duration of disease			0.374
<12 months	14 (37.8%)	12 (50.0%)	
12-36 months	10 (27.0%)	3 (12.5%)	
>36 months	13 (35.1%)	9 (37.5%)	
Mean±SD	38.84±47.65	49.75±67.654	0.463
Symptoms			
Swelling	36 (97.3%)	24 (100.0%)	0.416
Pain	14 (37.8%)	3 (12.5%)	**0.031**
Fever	1 (2.7%)	2 (8.3%)	0.320
Dyspnoea	2 (5.4%)	0 (0.0)	0.515
Palpitation	1 (2.7%)	2 (8.3%)	0.320
Pallor	0 (0.0%)	1 (4.2%)	0.211
Lymphadenopathy	0 (0.0%)	1 (4.2%)	0.211
Functional status			
Hyperthyroidism	1 (2.7%)	0 (0.0%)	0.266
Hypothyroidism	2 (5.4%)	4(16.6%)	
Euthyroidism	34 (91.9%)	20 (83.3%)	
Size of nodule (cm)			
≤5	12 (32.4%)	18(75.0%)	**0.001**
>5	25(67.6%)	6(25.0%)	
Mean ± SD	6.14±1.97	5.11±1.14	**0.023**
Nodularity			
STN	12 (32.4%)	18 (75.0%)	**0.001**
Multi-nodular	25 (67.6%)	6 (25.0%)	
Hemoglobin	11.94±1.46	11.6250±1.28376	0.380
T3	60.304±61.65	67.1071±79.02013	0.708
T4	13.42±15.22	12.7971±15.25253	0.877
TSH (Mean±SD)	1.92±0.94	2.73±1.74	**0.023**
Calcium level	8.94±0.94	9.33±1.10	0.145
Ultrasound findings			
Probably benign	32 (86.5%)	1 (4.2%)	**<0.001**
Suspiciously malignant	5 (13.5%)	23 (95.8%)	

VEGF expression was strongly positive (3+) in 26 (42.62%) patients and negative/equivocal (1+ & 2+) in 35 (57.38%) patients. Among all of patients, majority of patients (n=35) belong to weak to moderately positive for VEGF and rest (n=26) were strongly positive. In weak to moderately positive for VEGF, 15 (42.9%) patients were in TSH range from <1.39 mIU/liter and strongly positive in 3 (11.5%) patients. In TSH range 1.4-2.49 mIU/liter, 18 (51.4%) patients were weak to moderately positive for VEGF and 6 (23.1%) were strongly positive. While in TSH level >2.5 mIU/liter, only 2 (5.7%) patients were weak to moderately positive and 17 (65.4%) patients were strongly positive for VEGF showed significant association (p<0.001) (Table 2). On

comparing TSH level of benign and malignant patients individually with VEGF expression, significant association were also observed (p<0.001 and p=0.004 respectively) (Table 2).

Of 37 patients of benign group, follicular adenoma (Figure 1a, b) was the most common (n=4) lesion strongly positive for VEGF followed by solitary thyroid nodule, thyroiditis and adenomatous goiter (Figure 2a, b) of 2 patients each. In malignant group (n=24), papillary carcinoma (Figure 3a, b) was the most common (n=12) lesion strongly positive for VEGF followed by follicular and medullary carcinoma of 2 patients each. On comparing VEGV expression between benign and malignant group, 10 (27.0%) patients were strongly positive for VEGF whereas in malignant group, 16 (66.7%) patients were strongly positive for VEGF showed significant association (p=0.002) (Table 3).

Table 2: Correlation between age, nodule size, nodularity and TSH with VEGF expression

	VEGF		**p-value**
	1+ & +2 (Negative/Equivocal) (n=35)	**+3 (Positive) (n=26)**	
Age group (years)			
30-40	11 (31.4%)	8 (30.8%)	0.992
41-50	19 (54.3%)	14 (53.8%)	
>50	5 (14.3%)	4 (15.4%)	
Size of nodule (cm)			
≤5	18 (51.4%)	12 (46.2%)	0.683
>5	17 (48.6%)	14 (53.8%)	
Nodularity			
STN	14 (40.0%)	16 (61.5%)	0.096
Multi-nodular	21 (60.0%)	10 (38.5%)	
TSH (Benign n=37)			
<1.39	11 (40.7%)	2 (20.0%)	**<0.001**
1.4-2.49	14 (51.9%)	1 (10.0%)	
>2.5	2 (7.4%)	7 (70.0%)	
TSH (Malignant n=24)			
<1.39	4 (50.0%)	1 (6.2%)	**0.004**
1.4-2.49	4 (50.0%)	5 (31.2%)	
>2.5	0 (0.0%)	10 (62.5%)	
TSH (Total n=61)			
<1.39	15 (42.9%)	3 (11.5%)	**<0.001**
1.4-2.49	18 (51.4%)	6 (23.1%)	
>2.5	2 (5.7%)	17 (65.4%)	

Table 3: Correlation between benign and malignant histopathological features with VEGF expression

	VEGF		**p-value**
	1+ & +2 (Negative/Equivocal) (n=35)	**+3 (Positive) (n=26)**	
Histopathology (Benign (n=37)			
STN	10	2	0.325
MNG	1	0	0.999
Colloid Goitre	1	0	0.999
Hurthle cell Adenoma	1	0	0.999
Thyroiditis	1	2	0.106
Adenomatous goiter	3	2	0.482
Follicular adenoma	10	4	0.868
Histopathology (Malignant n=24)			
Papillary carcinoma	5	12	0.525
Follicular carcinoma	3	2	0.155
Medullary carcinoma	0	2	0.869
Histopathology (Total n=61)			
Benign	27	10	**0.002**
Malignant	8	16	

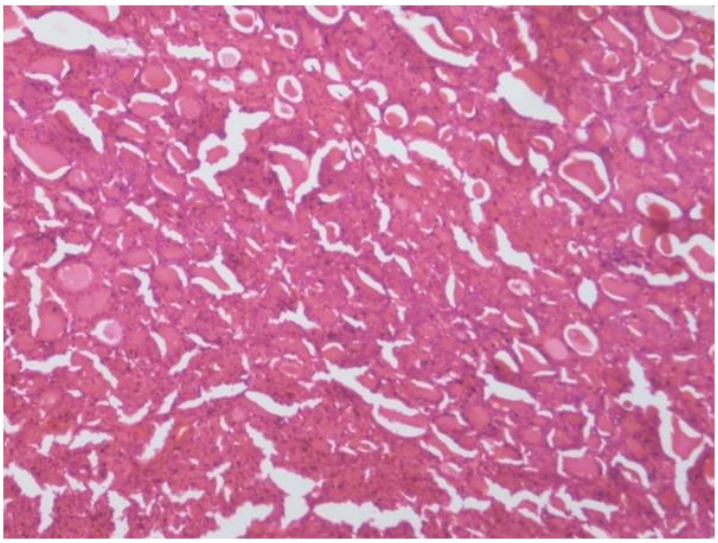

Figure 1a: H&E staining - Follicular adenoma

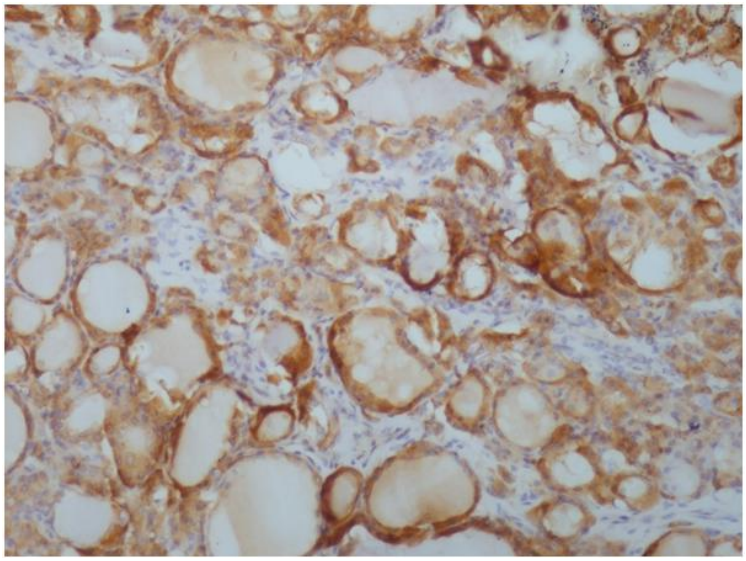

Figure 1b: IHC - Follicular adenoma showing moderate to strong cytoplasmic positivity (+3) for VEGF

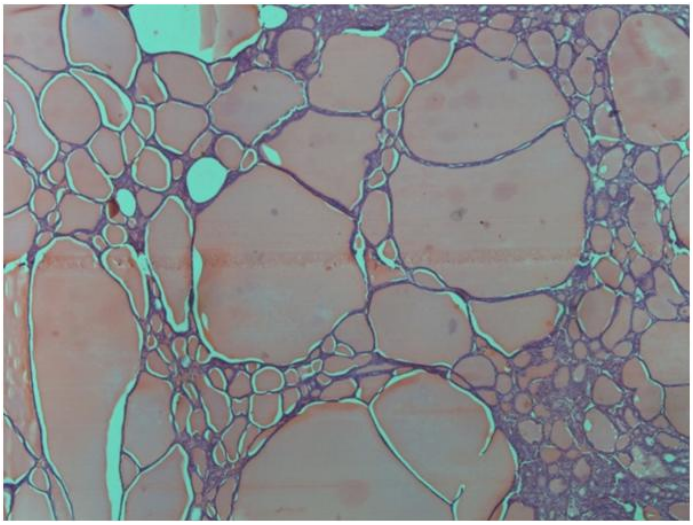

Figure 2a: H&E - Adenomatous goiter showing enlarged thyroid follicles filled with colloid

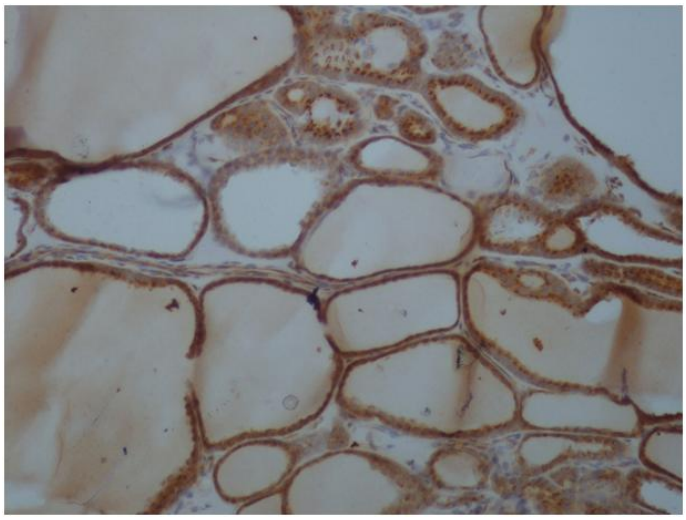

Figure 2b: IHC - Adenomatous goiter showing moderate to strong cytoplasmic positivity (+3) in many follicles

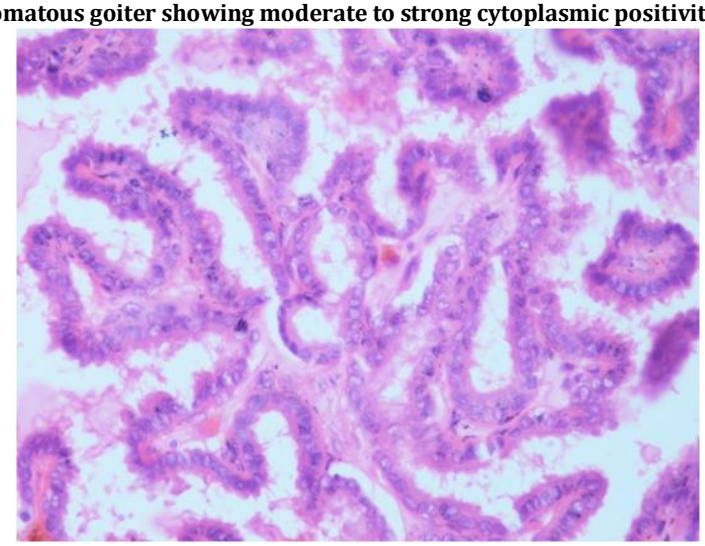

Figure 3a: H&E – Papillary thyroid carcinoma (PTC) showing papillae and nuclear features of PTC

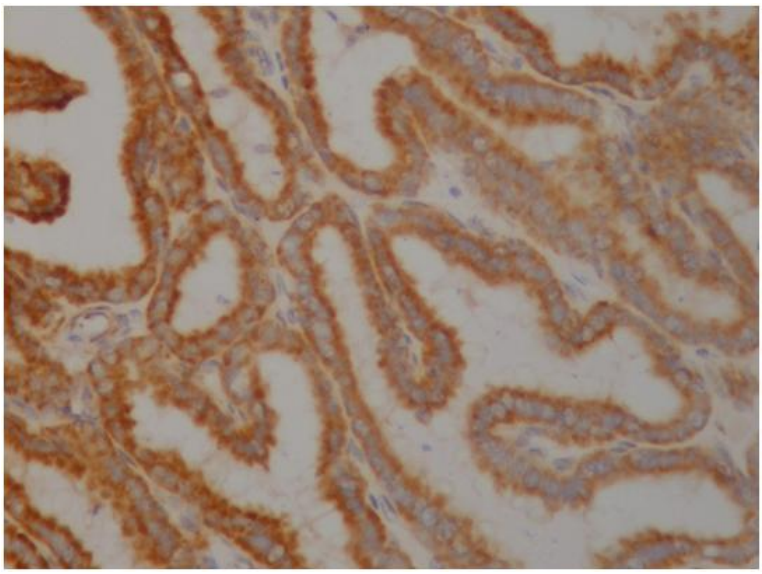

Figure 3b: IHC – Papillary thyroid carcinoma showing strong diffuse cytoplasmic positivity (+3) for VEGF

DISCUSSION

The aim of the present study is to the association between expression of VEGF and concentration of serum TSH in thyroid lesions (benign and malignant). In our study, the mean age of patients was 36.26±11.53 years (range 20-50 years) with female preponderance (n=50). In malignant group patients were younger than benign group (41.42±6.21 vs 47.27±6.35 years; p=0.001). It is consistent with a study done in India by Nagarkar et al [13].

As described, the average incidence of thyroid cancer observed in patients with hyperthyroidism in clinical trials ranged from 1.6 to 21.1 percent during surgery. [14]. It is very different from our study, which indicates that there is no patient with hyperthyroidism in the malignant group, while 4 (16.6%) patients were found to have symptoms with hypothyroidism and around 20 (83.3%) were euthyroid patients. Also within normal TSH levels a TSH level above the average population is associated with a substantially higher risk of thyroid cancer than a TSH below the average [15]. According to Welker et al [16], most patients with thyroid nodules are often considered to be euthyroid, with less than 1% of nodules that cause hyperthyroidism or malignant thyrotoxicosis.

In our study, solitary thyroid nodules were present in 12 (32.4%) patients and 25 (67.6%) patients in benign group were multi-nodular goiter, while in malignant group there were solitary thyroid nodules in 18 (75.0%) patients and 6 (25.0%) patients were multi-nodular goiter. The correlation between benign and malignant was found to be statistically significant (p<0.001). It is consistent with a study done by Lema et al [17].

In our study, 12 (32.4%) patients had nodule size < 5 cm and 25 (67.6%) patients had nodule size > 5 cm in benign group while 18 (75%) patients were nodule size < 5 cm and 6 (25.0%) patients were > 5 cm in malignant group. It was found that there was a statistically significant correlation between the size of the thyroid nodule and its existence (p<0.001) being benign or malignant. According to a study conducted by Nam-Goong et al[18], 8% of the 25 patients with incidentally observed thyroid nodules < 5 mm, 15% of the 153 patients with 5–10 mm nodules and 13% of the 139 patients with 10–15 mm nodules had cancer. The thyroid nodule size does not predict the risk of differentiated thyroid carcinoma.

High-resolution ultrasound with doppler color is key to thyroid lesion diagnosis. Over the past decade or two, a number of ultrasonic features have been established which help predict the risk of malignancy in a nodule. In our study, we found that in the histologically proven benign group, 32 (86.5%) patients were probably benign, and 5 (13.5%) patients were ultrasonographically suspiciously malignant, while in the histologically proven malignant group, 23 (95.8%) were suspiciously malignant, and only 1 case was probably benign. The correlation is considered to be statistically significant (p<0.001). According to Moon et al [19], the statistically significant features for the depiction of a benign nodule were an ovoid to round shape, a well-defined smooth margin, isoechogenicity, and a spongiform appearance and for a malignant nodule were a taller than wide shape, a spiculated margin, marked hypoechogenicity, microcalcification, and macrocalcification. Despite statistical significance, hypoechogenicity was found in 33.7% of benign nodules and 46.1% of malignant nodules.

Angiogenesis plays a key role in the development of both benign thyroid tissue and cancer of the thyroid. Numerous growth factors such as VEGF, FGF, PDFG derived from cancer cells and much more stimulate angiogenesis of the tumor by paracrine action. Between them, VEGF is the most essential and strongest growth factor for developing and metastasizing thyroid cancer. VEGF expression levels are often higher than normal in malignant tumours. The rate of VEGF expression in malignant lesions is associated with cancer incidence and progression [20,21]. The expression VEGF may be an indicator of the metastasis of localized and distant PTC [20].

Our study has shown that VEGF is overexpressed in malignant thyroid lesions compared with benign lesions (p=0.002), and is implicated in tumor growth and metastasis. Findings in the VEGF expression were consistent with other multiple studies [21,22]. Nevertheless, this study proposed that between benign and malignant lesions there was a difference in the expression of VEGF. Following our observations of overexpression of VEGF in thyroid disease including follicular adenoma (4/14 patients) and papillary thyroid cancer (12/17 patients), Jebreel et al [22] found both PTC (7/7 patients) and 94% (n=16) of multinodular goitre to be immunopositive to VEGF. In our study, we observed that VEGF was not significantly overexpressed in patients with age (>50 years), nodule size (>5 cm) and nodularity (multinodular) (P >0.05) that is consistent with a research conducted by Haytaoglu et al [23].

TSH is a main thyroid stimulating hormone, therefore, it determines various thyrocyte biological functions of thyrocyte and is recognized as a specific growth factor. We are drawn by the possible role that serum TSH can play in thyroid carcinoma. A elevated level of serum TSH in the presence of undiagnosed nodules is indicative of cancer and a strong association exists between serum TSH levels and tumor growth in PTC [6,24]. In summary, serum TSH plays a major role in thyroid cancer.

The mean levels of T3, T4 and serum calcium in both benign and malignant groups were comparable while the mean level of TSH in malignant group was significantly higher than in benign group (p=0.023) in our study. According to other study, there is considerable evidence that the serum TSH level is an independent marker for the diagnosis of thyroid malignancy in patients with nodular thyroid disease. In addition, preoperative serum TSH levels are elevated in patients with more aggressive tumours, indicating a possible role for TSH in differentiated thyroid cancer progression [24]. Several other studies identified as autonomously functioning thyroid nodules are less likely to have harbor malignancy [2, 25]. Accordingly, in those with TSH levels below the normal reference range, Boelaert et al [24] and others [5,6] find the lowest levels of thyroid malignancy, indicating the presence of thyroid autonomy [26].

The association between the serum TSH level and VEGF expression, however, has rarely been investigated. Human thyroid follicles have previously been documented to increase VEGF mRNA in response to TSH, and TSH induces VEGF secretion in cultures of human thyroid cells as well as in vitro thyroid cancer cells [27,28].

In thyroid cancer we observed a strong positive correlation between serum TSH levels and VEGF expression (p<0.001). Li et al [29] reported similar findings of serum TSH levels, which were positively associated with VEGF expression (p<0.01). We concluded that the serum TSH plays a significant role in thyroid cancer prevalence and growth. Thyroid tumors showing high serum TSH, especially > 2.5 mIU / L may be more aggressive, with higher probability of local infiltration and metastasis. Li et al [29] concluded that a positive correlation in PTC between preoperative serum TSH and tumor VEGF expression indicates that higher serum TSH leads to a more aggressive tumor phenotype.

The findings of our study mainly indicate that TSH and VEGF correlation has a more positive association in malignant thyroid lesions rather than benign lesions (p<0.001), which is consistent with Jen-Der Lin et al [30], they described in primary cultures that included normal human thyroid, medullary thyroid cancer, and papillary, follicular, and hurthle cell thyroid cancer cell lines, VEGF was secreted in significantly higher concentrations in all thyroid cancers compared to normal thyroid cells.

TSH stimulates both normal and neoplastic thyroid cells, and we have also indicated that serum VEGF should be further improved by stimulation of TSH [31]. Sato et al.[28] stated that the expression of VEGF messenger ribonucleic acid in vitro in thyroid epithelial cells was stimulated by TSH and thyroid stimulating antibodies. The VEGF then activated thyroid endothelial vascular cells, leading to increased blood vessels and thyroid volume.

In patients with untreated goitrous HT, Iitaha et al [32] found substantial association between serum TSH levels and the thyroid vascular index. Multiple studies showed an increase in thyroidal blood flow following endogenous or exogenous stimulation of TSH [33,34]. These findings indicate that TSH plays a significant role in thyroid angiogenesis.

In patients with persistent or recurrent thyroid cancer, VEGF expression will be substantially higher than in patients cured for the disease. In addition, since TSH promotes the growth of thyroid cancer cells and appears to directly increase the synthesis of VEGF in preclinical models, TSH stimulation may increase the circulating serum VEGF levels in patients with chronic or recurrent thyroid cancer [31].

Therefore correlation of TSH and VEGF is more positive in malignant group of patients rather than benign group, among malignant patients there is strong positivity is seen in papillary carcinoma of thyroid and in benign more positive correlation seen in follicular adenoma. The cause of discrepancy of our result is number of patients were too small so much larger local population based studies need to be conducted.

SOURCE OF FUNDING

None

CONFLICT OF INTEREST

None

REFERENCES

1. Unnikrishnan AG and Menon UV. (2011). Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol Metab; 15: S78–S81.
2. Hegedus L. (2004). Clinical practice. The thyroid nodule. NEJM;351:1764–1771.
3. Shi L, Li Y, Guan H, Li C, Shi L, Shan Z, Teng W. (2012). Usefulness of serum thyrotropin for riskprediction of differentiated thyroid cancers does not apply tomicrocarcinomas: result os 1,870 Chinese patients with thyroid nodules. Endocr J. 59(11):973–80
4. Jonklaas J, Nsouli-Maktabi H, Soldin SJ. (2008). Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. Thyroid. 18:943–952.

5. Polyzos SA, Kita M, Efstathiadou Z, Poulakos P, Slavakis A, Sofianou D, Flaris N, Leontsini M, Kourtis A, Avramidis A. (2008). Serum thyrotropin concentration as a biochemical predictor of thyroid malignancy in patients presenting with thyroid nodules. J Cancer Res Clin Oncol;134:953–960.
6. Haymart MR, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC, Chen H. (2008). Higher serum thyroidstimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroidcancer and advanced tumor stage. J Clin Endocrinol Metab ;93: 809–814.
7. Viglietto G, Maglione D, Rambaldi M et al.(1995). Upregulation of vascular endothelial growth factor (VEGF) and downregulation of placenta growth factor (PlGF) associated with malignancy in human thyroid tumors and cell lines. Oncogene; 11: 1569–179.
8. Soh EY, Duh QY, Sobhi SA et al. (1997). Vascular endothelial growth factor expression is higher in differentiated thyroid cancer than in normal or benign thyroid. J Clin Endocrinol Metab; 82: 3741–7.
9. Fenton C, Patel A, Dinauer C, Robie DK, Tuttle RM, Francis GL.(2000). The expression of vascular endothelial growth factor and the type 1 vascular endothelial growth factor receptor correlate with the size of papillary thyroid carcinoma in children and young adults. Thyroid; 10: 349–57.
10. Klein M, Vignaud JM, Hennequin V et al. (2001). Increased expression of the vascular endothelial growth factor is a pejorative prognosis marker in papillary thyroid carcinoma. J Clin Endocrinol Metab ; 86: 656–8.
11. Hoffmann S, Hofbauer LC, Scharrenbach V et al. (2004). Thyrotropin (TSH)-induced production of vascular endothelial growth factor in thyroid cancer cells in vitro. J Clin Endocrinol Metab; 89: 6139–45.
12. Klein M, Brunaud L, Muresan M et al.(2006). Recombinant human thyrotropin stimulates thyroid angiogenesis in vivo. Thyroid ; 16: 531–6.
13. Nagarkar R, Roy S, Akheel M, Palwe V, Kulkarni N, Pandit P.(2015). Incidence of Thyroid Disorders in India: An Institutional Retrospective Analysis. Int J Dent Med Spec ;2(2):19-23
14. Paschke R. (2016). Reasonable use of imaging tests for follow-up care of thyroid cancer. Dtsch Med Wochenschr. ;141(21):1521.
15. Megan Rist Haymart, Daniel John Repplinger, Glen E. Leverson, Diane F. Elson, Rebecca S. Sippel, Juan Carlos Jaume, Herbert Chen, (2008). Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. J Clin Endocrinol Metab. ;93(3):809–814.
16. Welker MJ, Orlov D. (2003). Thyroid nodules. Am Fam Physician. 67(3):559–566.
17. Lema LEK, Aziz MR, Mbembati NA, Mwakyoma HA. (1992). The frequency of carcinoma in solitary thyroid nodules and in multinodular goiters. East and Central African J Surg; 2:11-14.
18. Nam-Goong IS, Kim HY, Gong G, et al.(2004). Ultrasonography-guided fine-needle aspiration of thyroid incidentaloma: correlation with pathological findings. Clin Endocrinol. 60:21–8.
19. Moon WJ, Jung SL, Lee JH, et al. (2008). Benign and malignant thyroid nodules: US differentiation--multicenter retrospective study. Radiology.;247(3):762–770.
20. Klein M, Picard E, Vignaud JM et al. (1999). Vascular endothelial growth factor gene and protein: strong expression in thyroiditis and thyroid carcinoma. J Endocrinol; 161:41–9.
21. Katoh R, Miyagi E, Kawaoi A et al. (1999). Expression of vascular endothelial growth factor (VEGF) in human thyroid neoplasms. Hum Pathol 30: 891–7.
22. Jebreel A, England J, Bedford K, et al. (2007). Vascular endothelial growth factor (VEGF), VEGF receptors expression and microvascular density in benign and malignant thyroid diseases. Int J Exp Pathol ;88:271-7.
23. Haytaoglu G, Kuzu F, Arpaci D, Altas A, Can M, Barut F, et al. (2019). Correlation of vascular endothelial growth factor and vascular endothelial growth factor receptor-1 levels in serum and thyroid nodules with histopathological and radiological variables. J Lab Physicians 11:51-7.
24. Boelaert K, Horacek J, Holder RL et al.(2006). Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J Clin Endocrinol Metab , 91: 4295–301.
25. Hegedus L, Bonnema SJ, Bennedbaek FN. (2003). Management of simple nodular goiter: current status and future perspectives. Endocrine Reviews. 24:102–132.
26. Ross DS (2006). Predicting thyroid malignancy. J Clin Endocrinol Metab. 91: 4253–4255.
27. Soh EY, Sobhi SA, Wong MG et al. (1996). Thyroid-stimulating hormone promotes the secretion of vascular endothelial growth factor in thyroid cancer cell lines. Surgery;120: 944–7.
28. Sato K, Yamazaki K, Shizume K et al.(1995). Stimulation by thyroid-stimulating hormone and Grave's immunoglobulin G of vascular endothelial growth factor mRNA expression in human thyroid follicles in vitro and flt mRNA expression in the rat thyroid in vivo. J Clin Invest ; 96:1295–302.
29. Li J, Teng L, Jiang H. (2014). Relationship between preoperative serum TSH levels and expression of VEGF in papillary thyroid carcinoma. Asia Pac J Clin Oncol. 10(2):149–152.
30. Lin JD, Chao TC, Huang BY, Chen ST, Chang HY, Hsueh C. (2005). Thyroid cancer in the thyroid nodules evaluated by ultrasonography and fine-needle aspiration cytology. Thyroid. ;15(7):708–717.
31. Tuttle RM, Fleisher M, Francis GL, Robbins RJ. (2002). Serum vascular endothelial growth factor levels are elevated in metastatic differentiated thyroid cancer but not increased by short-term TSH stimulation. J Clin Endocrinol Metab ; 87: 1737–1742.
32. Iitaka M, Miura S, Yamanaka K, et al. (1998). Increased serum vascular endothelial growth factor levels and intrathyroidal vascular area in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab. ;83(11):3908–3912.

33. Tegler L, Gillquist J, Anderberg B, Jacobson G, Lundstrom B, Roos P. (1981). Human thyroid blood flow response to endogenous, exogenous human, and bovine thyrotrophin measured by electromagnetic flowmetry. Acta Endocrinol (Copenh). 98:540 –548.
34. Connors JM, Huffman LJ, Hedge GA. (1988). Effects of thyrotropin on vascular conductance of the thyroid gland. Endocrinology.122:921–929.