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Original Research Article

ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION BIOPSY RESULTS OF THYROID NODULES IN A SINGLE CENTER

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Abstract

Objective: To review the fine needle aspiration biopsy results of the thyroid (TFNAB) of 679 patients with nodular goiter who were diagnosed and followed in our center and to compare post-surgical diagnoses with pre-surgical TFNAB results in 27 patients. **Method:** Demographic and thyroid nodule characteristics on B-mode ultrasonography, TFNAB cytological findings and post-surgical histopathological results of all patients were retrospectively analyzed. **Results:** Mean age (±SD) of 679 patients during TFNAB was 53±12 years of whom 116 (17%) were males (mean age: 56±13.5 years) and 563 (83%) were females (mean age: 52.4±11.8 years). No significant difference was observed in terms of age between males and females. Of 679 patients, 614 (90%) were in euthyroid, 42 (6%) were hyperthyroid and 23 (4%) were hypothyroid. TFNAB was performed once in 592 (87%) patients, twice in 93 (12%) patients, and thrice in 8 (1%) patients. 27/679 patients with TFNAB (3.9%) underwent thyroidectomy due to suspected malignancy; the remaining 652 patients were followed-up by USG and periodic clinical examinations. According to the TFNAB-based diagnoses of 27 operated patients, 3 were classified as Bethesda 6, 3 as Bethesda 5, 11 as Bethesda 4 and 10 as Bethesda 3. Among the 27 patients, 10 were malignant and 17 were benign, according to the post-surgical histopathological analysis. Malignancy was diagnosed on the post-surgical histopathological analysis in all patients who were categorized as Bethesda 6 and 5. However, only 3 of the 11 patients who were classified as Bethesda 4 and 10 of the patients who were classified as Bethesda 3 were reported as malignant. Considering 10 patients who were diagnosed as malignant on the post-surgical histopathological result, the rate of malignancy was 1.5 in all 679. C**onclusion:** TFNAB results per se contribute to final diagnosis, particularly for nodules reported as Bethesda 5 and 6. However, in TFNAB results reported as Bethesda 3 and 4, cytological findings as well as clinical and USG features and if available, Elastosonographic or molecular genetics examinations should be taken into account for proper selection of patients for thyroidectomy.

Keywords: Fine needle aspiration biopsy of the thyroid (TFNAB), Bethesda classification, thyroid nodule, thyroid cancer

Introduction

The reported prevalence of thyroid nodules shows a great degree of variability between studies, ranging from 50% in postmortem examinations to up to 70% in high-resolution ultrasonography (USG) studies (1-3). In Western populations, the prevalence of thyroid nodules detected by palpation varies from 3% to 8% (4). In Turkey, the prevalence of thyroid nodules was reported as 2-6% and 18% by manual palpation and USG examination, respectively (5, 6). The widespread use of USG in recent years has led to an increased prevalence of thyroid nodules. For thyroid nodules, USG features suggestive of malignancy include irregular nodular margins, a dense, hypoechoic and solid structure, presence of internal microcalcification, and abnormal vascularisation within the module (7, 10). Thyroid fine needle aspiration biopsy (TFNAB) is considered as the most reliable diagnostic method for discrimination of benign and malignant thyroid nodules. Being a harmless, easy to perform and inexpensive method, TFNAB also offers reliable results and is a well-tolerated diagnostic method; therefore, nowadays TFNAB is a frequently preferred diagnostic tool (11-13). TFNAB is widely used for assessment of thyroid nodules since TFNAB results can provide more accurate information on the pathology of thyroid nodules when combined with other diagnostic methods (14,15). Thyroid cancer is the most common malignancy of the endocrine system and accounts for 1% of all cancers encountered in clinical practice (16). Although thyroid tumors usually grow slowly, they are associated with more deaths annually than all other endocrine cancers combined (11). The goal of thyroid surgery is to reduce the risk or presence of cancer through a proper indication and appropriate treatment method. TFNAB is considered the gold standard for preoperative assessment of thyroid nodules (17,18). Ultrasound-guided FNAB (UG-FNAB) has emerged as an alternative to conventional FNAB (C-FNAB) for the diagnostic evaluation of nonpalpable nodules and for the repeat evaluation of nodules with previous nondiagnostic C-FNAB. UG-FNAB is a biopsy procedure with greater diagnostic value compared to C-FNAB (19,20). Generally, TFNAB is the method of choice for solitary nodules or multinodular goiter when a nodule is greater than 1 cm and shows solid hypoechoic appearance with microcalcification on USG (15,20). TFNAB was reported to have a specificity ranging from 65% to 99%, and a specificity ranging from 72 to 100%, when used in the initial evaluation of thyroid nodules (21-23).

In the present study, we aimed to retrospectively analyze the TFNAB results of patients with thyroid nodules, followed-up at our center for at least 1 year, and post-surgical histopathological findings of patients who underwent total thyroidectomy.

Materials and Methods

Patients: In this study, the hospital computer records of 679 patients who underwent TFNAB and were followed-up between September 2014 and September 2015 at the Endocrinology and Metabolism Outpatient Clinic of our center were retrospectively analyzed.

Biopsy procedure: After a physical examination, the patient was kept in the supine position with hyperextension of the neck supported by a rolled pillow. The nodules were localized with a 10 MHz transducer prior to TFNAB. The overlying skin was cleaned with betadiene. Sampling was carried out by manual aspiration using a 22 gauge fine needle connected to a 10 mL syringe. Once the needle was inserted into the solid part of the nodule, negative syringe pressure was applied. After aspiration, smears were placed on glass slides and were air-dried. Two to three glass slides from each patient were stained with Wright-Giemsa stain to confirm the presence of follicular cells. The slides were subsequently

stained with May-Grünwald-Giemsa stain. Informed and signed consent was obtained from all patient. No serious complications were seen during the procedure. TFNAB results were classified into 6 groups according to the Bethesda classification (The Bethesda System for Reporting Thyroid Cytopathology Categories), in order to optimize diagnostic and therapeutic management of nodules (24). Furthermore, we compared USG finding and cytological result of TFNAB specimens in patients underwent thyroidectomy. Statistical analyses were performed by using x^2 test with Statistical Package for Social Sciences (SPSS for Windows, version 17; Chicago; IL). The results are presented as mean±standard deviation (SD), minimum and maximum levels. Sensitivity, specificity, positive and negative predictive values of the presurgical FNAB for final post-surgical histopathological results were counted.

Results

The mean age (±SD) of 679 patients undergoing TFNAB was 53±12 years (min:18, max:90) of whom 116 (17%) were males (mean age: 56±13.5 years; min:18, max:88) and 563 (83%) were females (mean age: 52.4±11.8 years; min:18, max:90). The number of female patients who were followed-up for a thyroid nodule was approximately five times higher than that of males. There was no significant difference in terms of age between males and females. Among 679 patients, 614 (90%), were euthyroid, while 42 (6%) and 23 (4%) hyperthyroidism and hypothyroidism, respectively.

According to thyroid USG findings, 353 (52%), 245 (36%), and 81 (12%) patients had a solitary 1, 2, 3 or more nodules, respectively. When the nodules were evaluated on the basis of USG echogenicity, 409 (60%), 73 (11%), 56 (8%) and 141 (21%) nodules were hypoechoic, isoechoic, hyperechoic and mixed pattern, respectively. According to USG results, 224 (33%) nodules had at least one suspicious feature for malignancy. Among them, 195 (29%) and 30 (4%) nodules showed a single and 2 suspicious findings, respectively. 455 (67%) did not have any USG feature suggestive of malignancy (**Table1**).

Table1: Demographic and nodular characteristics of patients diagnosed with nodular goiter

Nodular features of 679 patients		**Benign (n=669)**	**Malignant (n=10)**	***p***
Age		53±12	55.5±12.7	0.55
Gender	Male	114	2	0.15
	Female	557	6	
Thyroid function tests	Euthyroid	606	8	0.97
	Hyperthyroid	41	1	
	Hypothyroid	22	1	
Number of the nodules	One	350	3	0.15
	Two	243	2	
	More than two	76	5	
Echogenicity of the nodules	Hypoechoic	400	9	0.10
	Isoechoic	73	0	
	Hyperechoic	56	0	
	Mixed	140	1	
Mean diameter of the nodules	<1cm	43	4	0.16
	≥ 1cm	626	6	
Malignant ultrasonographic signs of the nodules	Present	218	6	0.88
	Absent	451	4	

Suspicious USG appearances in 195 cases including microcalcification, irregular nodular margins, increased intraocular vascularisation and presence of suspicious cervical lymph nodes were present in 52 (8%), 18 (3%), (17%) and 13 (2%) nodules, respectively. The average diameter of each nodule was obtained by calculating the arithmetical mean of all diameters of the nodule. The average diameter was 17.3±10.2 mm (min: 5.5, max: 94) for the first nodule, 13.2±6.3 mm (min: 5.3, max: 55.5) for the second nodule and 12.4±4.9 (min: 5, max: 27) mm for the third nodule.

TFNAB was performed on once and thrice 592 (87%), 93 (12%) and 8 (1%) patients, respectively. The rate of unsatisfactory diagnosis (Bethesda 1) was 15 and 12% among patients undergoing first and second TFNAB, respectively (**Table 2**).

Table 2: The number of TFNAB procedures performed and results as per Bethesda classification system

Bethesda Category	1st TFNAB results		2nd TFNAB results		3rd TFNAB results	
	Number	%	Number	%	Number	%
1	102	15	13	12	0	0
2	524	77	70	78	7	90
3	36	5	9	9	1	10
4	11	2	1	1	0	0
5	3	0.5	0	0	0	0
6	3	0.5	0	0	0	0
Total	679	100	93	100	8	100

Among patients underwent second TFNAB, 18, 74 and 1 were previously diagnosed as Bethesda 3, 1 and 4, respectively. Among patients underwent third TFNAB, second TFNAB results was Bethesda 3 and 1in three and five patients, respectively. In these 8 patients, only one of the 3rd TFNAB results was reported as Bethesda 3 and the other 7 results as Bethesda 2 (**Table 2**).

Among 679 patients with TFNAB, 27 (3.9%) were operated at our center due to suspected malignancy and the remaining 652 patients were followed by USG and clinical examinations. Among 27 operated patients, 10 were reported as having malignant nodules and 17 as benign nodules (**Table 3**).

Table 3: Characteristics of 27 patients who underwent total thyroidectomy

Features of 27 patients		**Benign (n=17)**	**Malignant (n=10)**	***p***
Age		51±17.3	55.5±12.7	0.38
Gender	Male	10	2	0.15
	Female	7	6	
Thyroid function tests	Euthyroid	9	8	0.10
	Hyperthyroid	1	1	
	Hypothyroid	7	1	
Number of the nodules	One	6	3	0.16
	Two	5	2	
	More than two	6	5	
Echogenicity of the nodules	Hypoechoic	7	9	0.10
	Isoechoic	6	0	
	Hyperechoic	1	0	
	Mixed	2	1	
Mean diameter of the nodules	<1cm	7	4	0.26
	≥ 1cm	10	6	
Malignant USG signs of the nodules	Present	13	6	0.61
	Absent	4	4	

Among 10 malignant cases, 9 were papillary carcinoma and 1 was anaplastic carcinoma. six of nine patients who were diagnosed as papillary carcinoma were categorized as classical variants and 3 as a follicular variant. Tumor diameter was less than 1 cm in 4 and between 1 and 4 cm in 6 cases with papillary carcinoma (**Table 4**).

Table 4: Demographic and nodular characteristics of 10 patients diagnosed with malignancy following total thyroidectomy

Patient no.	1	2	3	4	5	6	7	8	9	10
Age	72	69	43	42	63	68	40	55	42	61
Gender	M	M	F	F	F	F	F	F	F	F
TFT	Hyper	Eu	Hypo	Eu	Eu	Eu	Eu	Eu	Eu	Eu
Number of the nodules	2	1	3	3	1	2	3	Multinodular	Multinodular	1
Mean diameter of the nodules	50 mm	19 mm	20 mm	16 mm	26mm	21 mm	9 mm	8 mm	6 mm	9 mm
Echogenicity of the nodules	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hypoechoic	Hyperechoic
Malignant USG signs of the nodules	Absent	Absent	Absent	Abnormal intanodular vascularisation	Abnormal intanodular vascularisation	Absent	Microcalcification	Abnormal intanodular vascularisation	Irregular margin	Microcalcification
1st FNAB	Bethesda 6	Bethesda 6	Bethesda 5	Bethesda 5	Bethesda 6	Bethesda 5	Bethesda 3	Bethesda 4	Bethesda 4	Bethesda 3
2nd FNAB							Bethesda 3			Bethesda 3
3rd FNAB										
Postoperative diagnosis	Anaplastic thyroid carcinoma	Papillary carcinoma, classical variant	Papillary carcinoma, classical variant	Papillary carcinoma, classical variant	Papillary carcinoma, classical variant	Papillary carcinoma, classical variant	Papillary carcinoma, classical variant	Papillary carcinoma, follicular variant	Papillary carcinoma, follicular variant	Papillary carcinoma, follicular variant
Diameter of the malignant tumor	50 mm	12 mm	15 mm	14 mm	20 mm	11 mm	6 mm	3 mm	3 mm	6 mm

According to the TFNAB-based diagnoses of 27 operated patients, 3, 3, 11 and 4 patients were classified as Bethesda 6, 5, 4 and 3, respectively.

Preoperative TFNAB diagnosis of all 27 operated patients was Bethesda category 3 or greater; thus, TFNAB had a positive predictive value of 37%, negative predictive value of 100%, and the sensitivity of 100% and specificity of 50%, based on postoperative results. While all patients who were categorized as Bethesda 6 and 5 had a postoperative diagnosis of malignancy diagnosis, there were only 3 patients with malignant tumors each among 11 cases classified as Bethesda 4 and 10 as Bethesda 3.

Considering 10 patients with a postoperative diagnosis of malignancy, the malignancy rate was 1.5 for all 679 patients followed at our center.

Discussion

Major considerations for TFNAB for follow-up of thyroid nodules are to not overlook the risk of malignancy and also to avoid unnecessary surgery. Careful selection of patients with an increased risk of malignancy who are most suitable for surgical treatment intervention would avoid unnecessary surgical trauma and reduce the costs of surgery. Thus, a decision for thyroid surgery should be made by taking into account the USG features of thyroid nodules, as well as TFNAB results for in a patient who may have the highest probability of malignancy.

In the literature, the reported rate of malignancy ranges between 2.6% and 10.7% in patients undergoing thyroid surgery (25, 26). In the current study, thyroid cancer was detected in 1.5% (10/679) of 679 patients followed for thyroid nodules and in 37% (10/27) of surgically treated thyroidectomized patients. Given the substantial number of patients followed for thyroid nodules in the present study, the 1.5% malignancy rate observed is quite low, compared to that reported in the literature. This may be explained by the fact that rather than performing TFNAB in selected nodules with suspicious appearance, it was performed for in all thyroid nodules, irrespective of appearance, in order to not overlook the risk of malignancy. In the present study, no significant difference was observed between ultrasonographic appearances of malignant and benign thyroid nodules. This may have resulted from our decision to perform TFNAB for in a greater number of patients, even when their USG appearance was not suggestive of malignancy with the aim to exclude malignancy. Likewise, since our hospital is a tertiary health care facility, we may also have expanded indications for TFNAB further to exclude patients referred from other hospitals in the region with a request for TFNAB. Malignancy rate reported in the literature for patients undergoing thyroid surgery varies from 2.6% to 10.7% (25,26). The malignancy rate found among patients receiving thyroid surgery in our center is quite high compared to that reported in the literature. We have very strict surgical indications applied for patients selected for thyroid surgery. In our hospital, patients with thyroid diseases are followed in accordance with decisions taken by a council of endocrinologists and general surgeons, guided by the most recent guidelines and any decision to operate is made by a consensus. We established surgical indications based specifically on the results of TFNAB in patients considered for surgery in our hospital. TFNAB result was Bethesda 3 or greater higher for in all patients considered for surgery. to be surgically treated. Patients categorized initially as Bethesda 3 who had a 2nd TFNAB result still indicating Bethesda 3 were scheduled for thyroid surgery. In This way, we could avoid as much unnecessary surgery as possible. In the present study, all patients with initial TFNAB result showing Bethesda 5 or Bethesda 6 were diagnosed with thyroid malignancy. False negativity

negative results were not present these patients. However, among patients with a TFNAB results indicating Bethesda 3 or 4, malignancy was diagnosed in two patients from each category. In the literature, the reported rate of malignancy is 5-15% and 15-30% for Bethesda 3 and 4, respectively (27). In our study, based on 1st TFNAB results, 2 out of 36 (5.5%) patients reported as Bethesda 3, 2 out of 10 operated patients classified as Bethesda 3 (20%) and 2 out of 11 Bethesda 4 patients followed and operated (16.6%) were reported as malignant. Considering only those patients undergoing surgery, these malignancy rates can be considered as consistent with the lower limit of the range reported in the literature. Nevertheless, thyroid USG findings and information on other risk factors for thyroid cancer would be useful while evaluating surgical indications for Bethesda 3 and 4 patients. In our study, no difference was observed in USG characteristics of malignant and benign tumors as indicated by preoperative USG results. However, the ultrasound device used at our center for evaluation of thyroid nodules doesn't have elastographic measurement capability. If a device with such a capability had been used, then this examination would have provided additional data, particularly for patient categorized as Bethesda 3 and 4 (28). Deeper understanding of the molecular mechanisms involved in the development of thyroid cancer has paved the way for newer approaches to the diagnosis and treatment of thyroid cancers. Somatic mutations and other molecular alterations have been considered as useful prognostic predictors for the diagnosis of thyroid cancer and subsequently introduced to clinical practice. *BRAF* and *RAS* gene mutations, as well as RET/PTC and PAX8/PPARγ chromosomal rearrangements commonly found in thyroid cancer, have been delineated. Also, gene expression profile, epigenome-wide and genome-wide association, and single-nucleotide polymorphism (SNP) in microRNA genes, as well as other genes associated with thyroid carcinogenesis, have been demonstrated (29-32). Current guidelines recommend genetic testing for determination of thyroid cancer risk (33-35). However, genetic tests were not available at our center at the time the study was carried out. The need for genetic examinations particularly in patients categorized as Bethesda 3 or 4 based on TFNAB results was also evident in our study.

Conclusion: TFNAB results per se contribute to final diagnosis, particularly for nodules reported as Bethesda 5 and 6.

However, for TFNAB results reported as Bethesda 3 and 4, cytological findings as well as clinical and ultrasonographic features and possibly elastosonographic or molecular genetic examinations, if available, should be taken into account to select patients who are most suitable for surgery.

Conflict of interest: None.

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