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RESEARCH

Factors affecting the false negativity of fine-needle aspiration biopsy in thyroid nodules with indeterminate cytology

Belirsiz sitolojili tiroid nodüllerinde ince iğne aspirasyon biyopsisi yanlış negatifliğine etkili faktörler

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Abstract

Purpose: False negative results of fine needle aspiration biopsies (FNAB) remain one of the most important problems in the management of thyroid nodules. The aim of this study was to evaluate the factors affecting the false negative results of FNAB, especially in thyroid nodules with indeterminate cytology.

Materials and Methods: Patients with thyroid nodules who underwent FNAB and subsequent thyroidectomy between January 2017 and January 2020 were included in the study. FNAB was performed on suspicious nodules according to the TIRADS classification. Nodules with "atypia/follicular lesion of undetermined significance (AUS/FLUS)", "non-diagnostic (ND)" cytology or "follicular neoplasm/suspicious for follicular neoplasm (FN/SFN)" according to FNAB were defined as "indeterminate cytology (IC)". Nodules were analyzed in two groups. Nodules with false negative FNAB were included in Group-1, while nodules with true positive and true negative FNAB were included in Group-2. Demographic and clinical data, pathologic results and genetic profiles of the patients were statistically compared for all patients and IC group separately.

Results: The results of FNAB and final pathology were discordant in 94 patients (Group-1) and concordant in 233 patients (Group-2). In the IC subgroup, 56 of 95 patients were in IC/Group-1 and 39 patients were in IC/Group-2. The accuracy of FNAB was statistically significantly higher in the presence of extrathyroidal extension (71.4% vs. 30.8%), perineural/lymphovascular invasion (60.0% vs. 29.6%), classical variants (68.5% vs. 50.7%), non-encapsulated tumors (67.9% vs. 50.0%) and multicentricity (47.2% vs. 24.1%). In the IC group, the presence of thyroiditis (75.0% vs. 49.2%) and high serum Anti-TPO levels (60.0% vs. 30.7%) increased the rate of false

Öz

Amaç: İnce iğne aspirasyon biyopsilerinin (İİAB) yanlış negatif sonuçları tiroid nodüllerine yaklaşımda en önemli sorunlardan biri olmaya devam etmektedir. Bu çalışmanın amacı, özellikle belirsiz sitolojili tiroid nodüllerinde İİAB'nin yanlış negatif sonuçlarını etkileyen faktörleri değerlendirmektir.

Gereç ve Yöntem: Ocak 2017 ile Ocak 2020 arasında İİAB yapılan ve ardından tiroidektomi kararı alınan tiroid nodüllü hastalar çalışmaya dahil edildi. TIRADS sınıflamasına uygun olarak şüpheli nodüllerden İİAB yapıldı. İİAB'ye göre "atipi/önemi belirsiz foliküler lezyon (AUS/FLUS)", "Non-diagnostik sitoloji (ND)" veya "Folliküler neoplazm/foliküler neoplazm için şüpheli (FN/SFN)" olan nodüller "Belirsiz sitoloji (IC)" olarak tanımlandı. Nodüller İİAB ve final patoloji uyumlarına göre iki gruba ayrıldı. Yalancı negatif İİAB saptanan nodüller Grup-1'e alınırken, doğru pozitif ve doğru negatif İİAB sonuçları Grup 2'ye alındı. Hastaların demografik ve klinik verileri, patolojik sonuçları ve genetik profilleri tüm hastalar ve IC grubu için ayrı ayrı istatistiksel olarak karşılaştırıldı.

Bulgular: 94 hastanın İİAB sonuçları ile final patoloji sonucları uyumsuz (Grup-1), 233 hastanın ise uyumlu bulundu (Grup-2). IC alt grubunda 95 hastanın 56'sı IC/Grup-1'de, 39'u ise IC/Grup-2'de yer aldı. Ekstratiroidal yayılım (%71.4 vs. %30.8), perinöral/lenfovasküler invazyon (%60.0 vs. %29.6), klasik varyant varlığında (%68.5 vs. 50.7%), non-enkapsüle tümörlerde (%67.9 vs. %50.0) ve multisentrik tümörlerde (%47.2 vs. %24.1) İİAB doğruluğu istatistiksel olarak anlamlı derecede yüksek saptandı. IC grubunda tiroidit varlığında (%75.0 vs. %49.2) ve yüksek serum Anti-TPO düzeylerinde (%60.0 vs. %30.7) İİAB yanlış negatifliği artmaktadır. Yüksek serum Nötrofil/Lenfosit Oranı

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negativity on FNAB. High serum Neutrophil to Lymphocyte Ratio (NLR) and small tumor size also increase the false negativity of FNAB, especially in IC group patients.
Conclusion: The diagnostic accuracy of FNAB is increased in classical variant tumors and in tumors expected to be aggressive due to presence of extra-thyroidal extension, perineural/lymphovascular invasion, non-encapsulated tumors and multicentricity. It should be kept in mind that the false negative results of FNAB increase in the presence of thyroiditis, high level of serum anti-TPO and high level of NLR.
Keywords:. Thyroid nodule, FNAB, Indeterminate cytology, papillary thyroid carcinoma

(NLR) ve küçük tümör boyutu varlığı da özellikle IC grubu hastalarda İİAB'nin yanlış negatifliğini arttırmaktadır.
Sonuç: Ekstratiroidal yayılım, perinöral/lenfovasküler invazyon, non-enkapsüle tümör ve multisentrik tümörler gibi agresif seyir göstermesi beklenen tümörlerde ve klasik varyant tümörlerde İİAB'nin tanısal doğruluğu artmaktadır. Bununla beraber tiroidit varlığında, yüksek serum Anti-TPO düzeyleri ve yüksek NLR varlığında İİAB'nin yanlış negatif sonuçlarının arttığı akılda tutulmalıdır.

Anahtar kelimeler: Tiroid nodülü, İİAB, Indeterminate sitoloji, tiroid malignitesi

INTRODUCTION

The prevalence of nodules in thyroid gland is estimated to be 4-7% in general population[1]. However, nodules can be detected in 19-68% of asymptomatic patients who underwent thyroid ultrasonography (USG)[2]. Although most of the thyroid nodules are benign, malignant nodules can also be detected up to a rate of 15%[3]. Ultrasound-guided fine-needle aspiration biopsy (FNAB) has widely accepted as the most accurate method to evaluate the risk of malignancy in thyroid nodules[4]. Routine use of ultrasound guided FNAB has reduced the number of unnecessary thyroidectomies while increasing the detection rate of thyroid carcinomas[5]. However, cytological evaluation of FNAB results in "non-diagnostic" (ND) in 1.8-23.6% of patients, "atypia/significance undetermined follicular lesion" (AUS/FLUS) in 3-27.2% and "follicular neoplasm/suspicious for follicular neoplasm" (FN/SFN) in 2-25%. These subgroups have a malignancy risk up to 16.8-20%, 14-15.9% and 25%, respectively[6,7]. Current guidelines recommend that FNAB should be repeated after the initial ND or AUS/FLUS cytology result. Subsequent ND or AUS/FLUS results can be managed with thyroidectomy or close follow-up according to USG findings. Due to the variable risk of malignancy of thyroid nodules with "indeterminate cytology (IC)", the guidelines recommend to evaluate these nodules with diagnostic thyroidectomy. However, the majority (70-80%) of IC nodules are found to be benign in the final pathology report[8]. In addition to USG findings, molecular tests are also evaluated in the management of these nodules. However, the diagnostic contribution of these tests remains a controversial issue[9,10]. Anti-TPO immunostaining in addition to FNAB cytologic examination in suspicious nodules has also been found to increase the diagnostic accuracy[11].

False negative results of FNAB may increase due to the adequacy of the specimen, the number of sampled nodules, nodule size and the presence of a cystic component of the nodule or the presence of thyroiditis. The management of these nodules with the decision to follow-up or thyroidectomy remains controversial. The high false negative rate of FNAB leads to overtreatment and an increase in unnecessary thyroidectomies. The aim of this study was to identify laboratory and pathologic features such as the presence of thyroiditis and elevated anti-TPO levels that may affect the false negativity of FNAB and increase the rate of unnecessary thyroidectomy, especially in the IC subgroup of patients.

MATERIALS AND METHOD

Sample

This study was conducted in the general surgery clinic of İstanbul Medeniyet University, Goztepe Suleyman Yalcin City hospital. Between January 2017 and January 2020, patients who were admitted to our endocrine surgery outpatient clinic for thyroid nodule(s) and underwent FNAB followed by thyroidectomy were included in the study. Patients were evaluated by a pre/post-operative multidisciplinary council and thyroidectomy was performed by an experienced endocrine surgeon.

Procedure

A 23-gauge needle was used to perform FNAB under USG imaging of suspicious findings (e.g., presence of hypoechoic nodules, irregular nodule border,

presence of punctate calcifications, etc.) according to the "Thyroid Image Reporting and Data System (TIRADS)" classification on thyroid ultrasonography (USG). To improve the diagnostic accuracy of FNAB, FNAB with aspiration was performed at least 3 times with the patient lying in the supine position and the neck in hyperextension. Cytologic analysis was performed by experienced endocrine cytopathologists.

FNABs were performed according to TIRADS classification. FNAB was performed on TIRADS-3 nodules if the nodule size was >2.5 cm, on TIRADS-2 nodules if the nodule size was >1.5 cm, and finally on TIRADS-5 nodules if the nodule size was >0.5 cm. Nodules with "atypia/follicular lesion of undetermined significance (AUS/FLUS)", "non-diagnostic (ND)" cytology and "follicular neoplasm (FN) or suspected follicular neoplasm (SFN)" were defined as "indeterminate cytology (IC)".

Patients with AUS/FLUS or ND FNAB results underwent thyroidectomy if they had compression symptoms or if a repeat FNAB resulted again as ND or AUS/FLUS. Patients with "malignant" or "suspicious for malignancy (SM)" FNAB results and patients with "benign" FNAB results underwent thyroidectomy if they had compression symptoms or surgical indications for thyrotoxicosis. FNAB was performed by experienced radiologists in the interventional radiology unit under thyroid ultrasonography guidance.

Patients who have undergone thyroidectomy due to thyroid cancer or nodule, patients with diffuse goiter or nodules that did not meet the TIRADS criteria were excluded from the study. Likewise, patients who did not require thyroidectomy as a result of FNAB and did not have thyrotoxicosis or compression symptoms were also excluded from the study.

Data collection

Demographic characteristics, comorbidities, pathologic and genetic profiles of the patients were documented. In addition, thyroid hormone status, presence of autoimmune thyroiditis, presence of microcalcifications, nodule size and localization, and Neutrophil/Lymphocyte ratio were determined. Tumor size, variant, presence of lymphovascular and perineural invasion (LVI/PNI), extrathyroidal extension, presence of encapsulation and multicentricity were documented in patients with papillary cancer. Nodules were analyzed in two groups. Patients who were found to be benign or IC according to FNAB and malignant on final pathology were classified as "false negative" and formed Group-1. Patients whose FNAB results were consistent with the final pathology were included in Group-2. These two groups were statistically compared in terms of the factors that cause false negativity in FNAB. Subgroup analysis was also performed for patients with IC nodules.

Statistical analysis

In a similarly designed study, the false negative rate was found to be as 11.1%. Using this frequency, the minimum sample size required for this was study was calculated to be 312 people with a ±3 precision (https://www.openepi.com).

Descriptive statistics were presented as numbers with percentages (n, %) for categorical variables. For continuous variables, mean±standard deviation (SD) or median with minimum-maximum were used. Normal distribution was assessed with histograms, Q-Q plots and Shapiro-Wilk test. In group comparisons, categorical variables were compared with Pearson Chi-square test or Fisher's exact test. Mann-Whitney U test was used to compare continuous variables. Univariate logistic regression analysis was used to compute odds ratios and their 95% confidence interval. A p-value of <0.05 was considered statistically significant. Statistical analysis was done with R version 4.0.2 (https://www.r-project.org/),

This study was approved by the Scientific Ethics Committee of the Istanbul Medeniyet University, School of Medicine (decision no and date: 2018.0029 and 14.02.2018). This study was conducted in accordance with the principles of the declaration of Helsinki, and informed consent obtained from all of the participants.

RESULTS

Among 1855 patients who were admitted to our endocrine surgery outpatient clinic between 2017 and 2020 due to thyroid nodule(s), 327 patients who underwent thyroidectomy were included in the study. Among these patients, 65 (19.9%) were male and 262 (80.1%) were female with a mean age of 49.1±12.6 years. 240 patients had multi-nodular goiter and 87 patients had nodular goiter disease. In terms of hormone profile, 266 patients were euthyroid, 49 were hyperthyroid and 12 were hypothyroid. The

mean diameter of the nodules that underwent FNAB was 28.3±16.1 mm. Fourty (12.2%) nodules were located in the upper pole, 202 (61.8%) in the middle of the thyroid gland, 64 (19.6%) in the lower pole and 21 (6.42%) at the isthmus. Micro-calcifications and macro-calcifications were found in 26 (8.15%) and 43 (13.5%) nodules, respectively. FNAB was performed in 327 patients, 179 (54.7%) were benign, 20 (6.12%) were ND, 29 (8.87%) were AUS/FLUS, 46 (14.1%) were FN/SFN, 27 (8.26%) were SM and 26 (7.95%) were malignant (Table-1).

Table-1. Descriptive analysis of demographic, clinical and pathological data of patients.

Variable	**n (%)**	**N**
Age, mean±SD	49.1±12.6	327
Gender		327
-Male	65 (19.9%)	
-Female	262 (80.1%)	
Concomitant disease	85 (26.0%)	327
-Diabetes mellitus	38 (11.6%)	327
-Hypertension	70 (21.4%)	327
Presence of nodules		327
-Multi-nodular Goiter	240 (73.4%)	
-Nodular Goiter	87 (26.6%)	
Thyroid function		327
-Euthyroidism	266 (81.3%)	
-Hyperthyroidism	49 (15.0%)	
-Hypothyroidism	12 (3.7%)	
Presence of thyroiditis	111 (33.9%)	327
Serum TSH level		327
-Normal	266 (81.3%)	
-Low	49 (15.0%)	
-High	12 (3.7%)	
Serum T3 level		326
-Normal	314 (96.3%)	
-Low	2 (0.6%)	
-High	10 (3.1%)	
Serum T4 level		327
-Normal	314 (96.0%)	
-Low	8 (2.5%)	
-High	5 (1.5%)	
Serum Anti-TPO level		103
-Normal	88 (85.4%)	
-High	15(14.6%)	
Serum Anti-Thyroglobulin level		96
-Normal	74 (77.1%)	
-High	22 (22.9%)	
Diameter of the nodule performed FNAB (mm), mean±SD	28.3±16.1	327
Location of the nodule performed FNAB		327
-Upper pole	40 (12.2%)	
-Middle of thyroid	202 (61.8%)	
-Lower pole	64 (19.6%)	
-Isthmus	21 (6.4%)	
Presence of calcification		319
-None	250 (78.4%)	
-Micro-calcification	26 (8.1%)	
-Macro-calcification	43 (13.5%)	
FNAB results		
-Benign	179 (54.7%)	
-Non-diagnostic (ND)	20 (6.1%)	
-Atypia/follicular lesion of undetermined significance (AUS/FLUS)	29 (8.9%)	
-Follicular neoplasm or suspicious for follicular neoplasm (FN/SFN)	46 (14.1%)	
-Suspicious for malignancy (SM)	27 (8.3%)	

-Malign	26 (7.9%)	
Final pathology		
-Benign -Malign	187 (57.2%) 140 (42.8%)	
Type of thyroid cancer		140
-Papillary cancer	131 (93.6%)	
-Medullary cancer	3 (2.1%)	
-Folliculary cancer	6 (4.3%)	
Malignant nodule diameter (mm), mean±SD	14.7±12.1	140
Presence of encapsulation	62 (44.3%)	140
Variant type		131
-Classic variant	73 (55.7%)	
-Other variants	58 (44.3%)	
Follicular variant	43 (74.1%)	
Oncocytic variant	8 (13.8%)	
Tall cell variant	4 (6.9%)	
Whartin like variant	2 (3.5%)	
Solid variant	1 (1.7%)	
Presence of lymphovascular/perineural invasion	15 (10.7%)	140
Extra-thyroidal extension	7 (5.0%)	140
Intra-thyroidal extension	12 (8.5%)	140
Presence of multicentricity	53 (37.9%)	140
Presence of K-ras mutation	14 (26.9%)	52
Presence of N-ras mutation	4 (8.9%)	45
Presence of BRAF mutation	12 (21.4%)	56
White blood cell, mean±SD	7.40±1.90	317
-Neutrophil	4.19±1.48	317
-Lymphocyte	2.42±0.79	317
-Neutrophil / Lymphocyte Ratio (NLR)	1.93±1.21	317

SD: Standard deviation, **TSH**: Thyrotropin releasing hormone, **Anti-TPO**: Anti thyroid peroxidase antibody, **FNAB**: Fine-needle aspiration biyopsy.

According to the final pathology results, malignancy was detected in 140 (42.8%) patients. The mean tumor diameter was found to be 14.7±12.1 mm in 140 patients. Encapsulation, classical variant and other variants were detected in 62 (44.3%), 73 (55.7%) and 58 (44.3%) patients, respectively. Lymphovascular invasion, extra-thyroidal extension, multi-centricity was detected in 15 (10.7%), 7 (5%) and 53 (37.9%) patients, respectively. According to genetic tests K-ras, N-ras and BRAF mutations were detected in 14 (26.9%), 4 (8.89%) and 12 (21.4%) patients, respectively (Table-1).

FNAB results were accepted to be false negative in 94 of 327 patients (Group-1) with malignancy on final pathology. Of the 94 patients, 38 had benign, 7 had ND, 14 had AUS/FLUS and 35 had FN/SFN results according to FNAB. On the other hand, FNAB results were consistent with the final pathology in 233 patients (Group-2). Patients with false negative FNAB results were statistically compared with patients with concordant FNAB and final pathology results. There was no difference between the two groups in terms of age, gender and comorbidity. There was no difference between the groups in terms of hormonal activity status, TSH, T3, T4 and Anti-thyroglobulin values, but Anti-TPO elevation was significantly higher in Group-1 (30.7% vs. 60.0%, p:0.027). Although the presence of thyroiditis was higher in Group-1, there was no statistically significant difference between the groups (25.5% vs. 35.1%, p:0.067) (Table-2).

Nodule diameter, nodule location, and the presence of micro/macro calcifications did not affect the false negativity of FNAB. False-negative FNAB results were more common in the presence of encapsulation, intra-thyroid extension and non-classical variants of papillary carcinoma, but statistically significant results were not found (p>0.05). On the other hand, false-negative FNAB results were statistically significantly less common in the presence of LVI/PNI (70.4% vs. 40.0%, p:0.038), extra-thyroidal extension (69.2% vs. 28.6%, p:0.039) and multicentricity (75.9% vs. 52.8%, p:0.009) (Table-2). There was no statistically significant difference between the groups in terms of the presence of K-ras, N-ras and BRAF mutations or

the Neutrophil/Lymphocyte ratio (NLR) of the patients.

Table-2. Comparison of demographic, clinical and pathological outcomes of patients between groups.

	Group-2	Group-1	p	OR (95% CI)c	N
	n=233	n=94			
Age, mean±SD	49.0±11.7	49.1±14.5	0.941a	1.00 (0.98-1.02)	327
Gender			0.329b		327
-Male	50 (76.9%)	15 (23.1%)		Ref.	
-Female	183 (69.8%)	79 (30.2%)		1.43 (0.77-2.78)	
Concomitant disease	60 (70.6%)	25 (29.4%)	0.985b	1.05 (0.60-1.79)	327
-Diabetes mellitus	25 (65.8%)	13 (34.2%)	0.548b	1.34 (0.63-2.72)	327
-Hypertension	49 (70.0%)	21 (30.0%)	0.910b	1.08 (0.60-1.92)	327
Presence of nodules			0.332b		327
-Multi-nodular Goiter	167 (69.6%)	73 (30.4%)		Ref.	
-Nodular Goiter	66 (75.9%)	21 (24.1%)		0.73 (0.41-1.27)	
Thyroid function			0.636b		327
-Euthyroidism	192 (72.2%)	74 (27.8%)		Ref.	
-Hyperthyroidism	32 (65.3%)	17 (34.7%)		1.38 (0.71-2.62)	
-Hypothyroidism	9 (75.0%)	3 (25.0%)		0.89 (0.18-3.16)	
Presence of thyroiditis			0.067b		327
-No	161 (74.5%)	55 (25.5%)		Ref.	
-Yes	72 (64.9%)	39 (35.1%)		1.58 (0.96-2.60)	
Serum TSH level			0.636b		327
-Normal	192 (72.2%)	75 (27.8%)		Ref.	
-Low	32 (65.3%)	16 (34.7%)		1.38 (0.71-2.62)	
-High	9 (75.0%)	3 (25.0%)		0.89 (0.18-3.16)	
Serum T3 level			0.334b		326
-Normal	226 (72.0%)	88 (28.0%)		Ref.	
-Low or high	7 (58.3%)	5 (41.7%)		1.85 (0.52-6.08)	
Serum T4 level			0.886b		327
-Normal	224 (71.3%)	90 (28.7%)		Ref.	
-Low	6 (75.0%)	2 (25.0%)		0.87 (0.11-4.00)	
-High	3 (60.0%)	2 (40.0%)		1.69 (0.19-11.3)	
Serum Anti-TPO level			0.027*b		103
-Normal	61 (69.3%)	27 (30.7%)		Ref.	
-High	6 (40.0%)	9 (60.0%)		3.32 (1.07-11.0)	
Serum Anti-Thyroglobulin level			0.975b		96
-Normal	48 (64.9%)	26 (35.1%)		Ref.	
-High	15 (68.2%)	7 (31.8%)		0.87 (0.29-2.37)	
Diameter of the nodule performed FNAB (mm), median (min-max)	28.0 (5.0-90.0)	21.0 (5.0-70.0)	0.313a	0.99 (0.98-1.01)	327
Location of the nodule performed FNAB			0.963b		327
-Upper pole	28 (70.0%)	12 (30.0%)		Ref.	
-Middle of thyroid	145 (71.8%)	57 (28.2%)		0.91 (0.44-1.99)	
-Lower pole	46 (71.9%)	18 (28.1%)		0.91 (0.38-2.23)	
-Isthmus	14 (66.7%)	7 (33.3%)		1.17 (0.36-3.66)	
Presence of calcification			0.946b		319
-None	179 (71.6%)	71 (28.4%)		Ref.	
-Micro-calcification	18 (69.2%)	8 (30.8%)		1.13 (0.44-2.66)	
-Macro-calcification	30 (69.8%)	13 (30.2%)		1.10 (0.52-2.20)	
Type of thyroid cancer			0.311b		140
-Papillary cancer	43 (32.8%)	88 (67.2%)		Ref.	

-Medullary cancer	2 (66.7%)	1 (33.3%)		0.26 (0.01-3.32)	
-Follicular cancer	1 (16.7%)	5 (83.3%)		2.20 (0.32-59.4)	
Malignant nodule diameter (mm), median (min-max)	10.0 (2.0-35.0)	12.0 (1.0-55.0)	0.758a	1.02 (0.99-1.05)	140
Presence of encapsulation			0.078b		140
-No	31 (39.7%)	47 (60.3%)		Ref.	
-Yes	15 (24.2%)	47 (75.8%)		2.05 (0.99-4.39)	
Variant type			0.104b		131
-Classic variant	29 (39.7%)	44 (60.3%)		Ref.	
-Other variants	14 (24.2%)	44 (75.8%)		1.92 (0.94-4.04)	
Presence of lymphovascular/perineural invasion			0.038*b		140
-No	37 (29.6%)	88 (70.4%)		Ref.	
-Yes	9 (60.0%)	6 (40.0%)		0.29 (0.09-0.86)	
Extra-thyroidal extension			0.039*b		140
-No	41 (30.8%)	92 (69.2%)		Ref.	
-Yes	5 (71.4%)	2 (28.6%)		0.19 (0.02-0.96)	
Intra-thyroidal extension			0.060b		140
-No	39 (30.5%)	89 (69.5%)		Ref.	
-Yes	7 (58.3%)	5 (41.7%)		0.32 (0.09-1.08)	
Presence of multicentricity			0.009*b		140
-No	21 (24.1%)	66 (75.9%)		Ref.	
-Yes	25 (47.2%)	28 (52.8%)		0.36 (0.17-0.75)	
K-ras mutation			0.689b		52
-Negative	15 (39.5%)	23 (60.5%)		Ref.	
-Positive	4 (28.6%)	10 (71.4%)		1.59 (0.43-6.92)	
N-ras mutation			>0.999b		45
-Negative	15 (36.6%)	26 (63.4%)		Ref.	
-Positive	1 (25.0%)	3 (75.0%)		1.59 (0.17-48.4)	
BRAF mutation			0.313b		56
-Negative	14 (31.8%)	30 (68.2%)		Ref.	
-Positive	6 (50.0%)	6 (50.0%)		0.47 (0.12-1.81)	
White blood cell, median (min-max)	7.3 (3.9-16.9)	7.2 (4.1-12.6)	0.292a	0.92 (0.81-1.05)	317
-Neutrophil, median (min-max)	4.0 (1.4-10.9)	4.2 (1.6-10.0)	0.608a	0.98 (0.83-1.15)	317
-Lymphocyte, median (min-max)	2.4 (0.4-6.2)	2.3 (0.9-4.5)	0.375a	0.80 (0.59-1.10)	317
-Neutrophil / Lymphocyte Ratio (NLR), median (min-max)	1.7 (0.3-15.2)	1.7 (0.7-7.7)	0.669a	1.08 (0.90-1.31)	317

[a]Mann-Whitney U test, [b]Chi-square test, [c]Odds ratio, 95% confidence interval (univariate logistic regression), *p<0.05
OR: Odds ratio, **SD**: Standard deviation, **TSH**: Thyrotropin releasing hormone, **Anti-TPO**: Anti thyroid peroxidase antibody, **FNAB**: Fine-needle aspiration biyopsy.

Subgroup analysis was performed for patients with IC nodules according to FNAB. Patients with malignancy on final pathology were defined as the false negative FNAB group (IC/Group-1) and statistically compared with patients who had benign nodules on final pathology (IC/Group-2) (Table-3). Demographic, clinical and laboratory data were similar between the two groups. However, we found that the false negative results of FNAB were statistically significantly higher in patients with thyroiditis compared to patients without thyroiditis (75% vs. 49.2%, p:0.023). This subgroup analysis revealed that the smaller tumor size, relevant with the higher FNAB false negativity (p:0.003). When NLR rates were compared between groups, it was found that NLR was statistically higher in IC/Group-1 (1.8 vs. 1.6, p:0.047) (Table-3).

Table-3. Comparison of demographic, clinical and pathological outcomes of patients with "Indeterminate Cytology " in terms of false negativity of FNAB.

Patients with Indeterminate Cytology	IC/Group-2	IC/Group-1	p	OR (95% CI)[c]	N
	n=39	n=56			
Age, mean±SD	52.3±11.8	48.7±14.6	0.250[a]	0.98 (0.95-1.01)	95
Gender			0.086[b]		95
-Male	13 (59.1%)	9 (40.9%)		Ref.	
-Female	26 (35.6%)	47 (64.4%)		2.57 (0.97-7.11)	
Concomitant disease	10 (38.5%)	16 (61.5%)	0.935[b]	1.15 (0.46-3.01)	95
-Diabetes mellitus	5 (35.7%)	9 (64.3%)	0.884[b]	1.28 (0.40-4.62)	95
-Hypertension	6 (31.6%)	13 (68.4%)	0.498[b]	1.64 (0.57-5.18)	95
Presence of nodules			0.509[b]		95
-Multi-nodular Goiter	29 (38.7%)	46 (61.3%)		Ref.	
-Nodular Goiter	10 (50.0%)	10 (50.0%)		0.63 (0.23-1.75)	
Thyroid function			0.756[b]		95
-Euthyroidism	35 (42.2%)	48 (57.8%)		Ref.	
-Hyperthyroidism/ Hypothyroidism	4 (33.3%)	8 (66.7%)		1.43 (0.41-5.92)	
Presence of thyroiditis			0.023*[b]		95
-No	30 (50.8%)	29 (49.2%)		Ref.	
-Yes	9 (25.0%)	27 (75.0%)		3.04 (1.25-7.96)	
Serum TSH level			>0.999[b]		95
-Normal	35 (41.7%)	49 (58.3%)		Ref.	
-Low/High	4 (36.4%)	7 (63.6%)		1.23 (0.33-5.20)	
Serum T3 level			0.646[b]		94
-Normal	36 (40.4%)	53 (59.6%)		Ref.	
-High	3 (60.0%)	2 (40.0%)		0.47 (0.05-3.21)	
Serum T4 level			>0.999[b]		95
-Normal	38 (41.3%)	54 (58.7%)		Ref.	
-Low	1 (33.3%)	2 (66.7%)		1.32 (0.10-42.5)	
Serum Anti-TPO level			0.309[b]		34
-Normal	8 (27.6%)	21 (72.4%)		Ref.	
-High	0 (0.00%)	5 (100%)		-[d]	
Serum Anti-Thyroglobulin level			0.632[b]		32
-Normal	5 (20.0%)	20 (80.0%)		Ref.	
-High	2 (28.6%)	5 (71.4%)		0.62 (0.09-5.92)	
Diameter of the nodule performed FNAB (mm), median (min-max)	30.0 (6.0-90.0)	18.0 (5.0-70.0)	0.003*[a]	0.97 (0.94-0.99)	95
Location of the nodule performed FNAB			0.076[b]		95
-Upper pole	1 (8.3%)	11 (91.7%)		Ref.	
-Middle of thyroid	26 (48.1%)	28 (51.9%)		0.11 (0.00-0.65)	
-Lower pole	9 (40.9%)	13 (59.1%)		0.15 (0.01-1.04)	
-Isthmus	3 (42.9%)	4 (57.1%)		0.15 (0.00-1.66)	
Presence of calcification			0.754[b]		93
-None	33 (42.3%)	45 (57.7%)		Ref.	
-Micro-calcification	1 (20.0%)	4 (80.0%)		2.64 (0.35-74.6)	
-Macro-calcification	4 (40.0%)	6 (60.0%)		1.09 (0.28-4.73)	
Variant type			-		56
-Classic variant	-	23 (41.1%)		-	
-Other variants	-	33 (58.9%)		-	
Folicullar variant	-	24 (72.7%)		-	
Oncocytic variant	-	7 (21.2%)		-	
Tall cell variant	-	2 (6.1%)		-	
K-ras mutation			0.318[b]		22
-Negative	0 (0%)	15 (100%)		Ref.	
-Positive	1 (14.3%)	6 (85.7%)		-[d]	
N-ras mutation			>0.999[b]		19
-Negative	1 (5.9%)	16 (94.1%)		Ref.	

-Positive	0 (0%)	2 (100%)		-[d]	
BRAF mutation			>0.999[b]		24
-Negative	1 (5.3%)	18 (94.7%)		Ref.	
-Positive	0 (0%)	5 (100%)		-[d]	
White blood cell, median (min-max)	6.4 (4.6-13.5)	7.2 (4.1-11.5)	0.742[a]	0.99 (0.80-1.22)	95
-Neutrophil, median (min-max)	3.5 (1.7-9.7)	4.2 (2.2-7.6)	0.261[a]	1.10 (0.84-1.44)	95
-Lymphocyte, median (min-max)	2.2 (1.2-4.2)	2.2 (0.9-4.0)	0.307[a]	0.68 (0.39-1.18)	95
-Neutrophil / Lymphocyte Ratio (NLR), median (min-max)	1.6 (0.9-3.4)	1.8 (0.8-6.3)	0.047*[a]	1.69 (0.99-2.86)	95

[a]Mann-Whitney U test, [b]Chi-square test, [c]Odds ratio (univariate logistic regression), [d]Can not be calculated due to very few observations in the groups, *p<0.05

IC: Indeterminate cytology, **OR**: Odds ratio, **SD**: Standard deviation, **TSH**: Thyrotropin releasing hormone, **Anti-TPO**: Anti thyroid peroxidase antibody, **FNAB**: Fine-needle aspiration biyopsy.

In another subgroup analysis, patients with IC with a final pathology of malignant (false negative) were compared with patients with malignant FNAB results (true positive) (Table-4). FNAB false negativity was higher in the presence of encapsulated tumors (50.0% vs. 32.1%, p:0.048) and non-classical variants of papillary carcinoma (50% vs. 30.1%, p:0.032) (Table-4).

Table-4. Comparison of the malign nodules in patients with "Indeterminate Cytology (IC)" according to FNAB with other malign nodules detected with FNAB.

	IC nodules with malign final pathology (False-Negative)	**Other malign nodules (True-Positive)**	**p**	**OR (95% CI)[c]**	**N**
	n=56	**n=84**			
Age, mean±SD	48.7±14.6	47.8±13.2	0.628[a]	1.00 (0.97-1.02)	140
Gender			0.805[b]		140
-Male	9 (45.0%)	11 (55.0%)		Ref.	
-Female	47 (39.2%)	73 (60.8%)		1.27 (0.47-3.34)	
Concomitant disease	16 (41.0%)	23 (59.0%)	>0.999[b]	0.94 (0.44-2.03)	140
-Diabetes mellitus	9 (47.4%)	10 (52.6%)	0.650[b]	0.71 (0.26-1.93)	140
-Hypertension	13 (40.6%)	19 (59.4%)	>0.999[b]	0.96 (0.43-2.21)	140
Presence of nodules			0.763[b]		140
-Multi-nodular Goiter	46 (41.1%)	66 (58.9%)		Ref.	
-Nodular Goiter	10 (35.7%)	18 (64.3%)		1.25 (0.53-3.06)	
Thyroid function			0.669[b]		140
-Euthyroidism	48 (41.7%)	67 (58.3%)		Ref.	
-Hyperthyroidism	6 (30.0%)	14 (70.0%)		1.64 (0.61-5.02)	
-Hypothyroidism	2 (40.0%)	3 (60.0%)		1.05 (0.15-9.32)	
Presence of thyroiditis			0.652[b]		140
-No	29 (37.7%)	48 (62.3%)		Ref.	
-Yes	27 (42.9%)	36 (57.1%)		0.81 (0.41-1.60)	
Serum TSH level			0.387[b]		140
-Normal	49 (42.2%)	67 (57.8%)		Ref.	
-Low	5 (26.3%)	14 (73.7%)		2.00 (0.70-6.69)	
-High	2 (40.0%)	3 (60.0%)		1.07 (0.16-9.51)	
Serum T3 level			>0.999[b]		139
-Normal	53 (39.8%)	80 (60.2%)		Ref.	
-Low/High	2 (33.3%)	4 (66.7%)		1.28 (0.23-10.6)	
Serum T4 level			0.494[b]		140
-Normal	54 (40.0%)	81 (60.0%)		Ref.	
-Low/High	2 (40.0%)	3 (60.0%)		0.98 (0.14-8.64)	
Serum Anti-TPO level			0.624[b]		50
-Normal	21 (55.3%)	17 (44.7%)		Ref.	
-High	5 (41.7%)	7 (58.3%)		1.70 (0.45-6.89)	
Serum Anti-Thyroglobulin level			0.554[b]		47

-Normal	20 (57.1%)	15 (42.9%)		Ref.	
-High	5 (41.7%)	7 (58.3%)		1.83 (0.48-7.53)	
Diameter of the nodule performed FNAB (mm), median (min-max)	18.0 (5.0-70.0)	15.0 (5.0-64.0)	0.203[a]	0.99 (0.97-1.02)	140
Location of the nodule performed FNAB			0.198[b]		140
-Upper pole	11 (47.8%)	12 (52.2%)		Ref.	
-Middle of thyroid	28 (34.1%)	54 (65.9%)		1.76 (0.68-4.57)	
-Lower pole	13 (56.5%)	10 (43.5%)		0.71 (0.22-2.31)	
-Isthmus	4 (33.3%)	8 (66.7%)		1.78 (0.42-8.63)	
Presence of calcification			0.172[b]		138
-None	45 (44.6%)	56 (55.4%)		Ref.	
-Micro-calcification	4 (25.0%)	12 (75.0%)		2.34 (0.75-9.12)	
-Macro-calcification	6 (28.6%)	15 (71.4%)		1.97 (0.73-6.02)	
Type of thyroid cancer			0.359[b]		140
-Papillary cancer	51 (38.9%)	80 (61.1%)		Ref.	
-Medullary cancer	1 (33.3%)	2 (66.7%)		1.20 (0.09-38.3)	
-Follicular cancer	4 (66.7%)	2 (33.3%)		0.33 (0.04-1.88)	
Malignant nodule diameter (mm), median (min-max)	13.0 (1.0-52.0)	10.0 (1.0-55.0)	0.280[a]	0.99 (0.96-1.02)	140
Presence of encapsulation			**0.048*** [b]		140
-No	25 (32.1%)	53 (67.9%)		Ref.	
-Yes	31 (50.0%)	31 (50.0%)		0.48 (0.24-0.94)	
Variant type			**0.032*** [b]		131
-Classic variant	22 (30.1%)	51 (69.9%)		Ref.	
-Other variants	29 (50%)	29 (50%)		0.48 (0.24-0.95)	
Presence of lymphovascular/perineural invasion			0.780[b]		140
-No	51 (40.8%)	74 (59.2%)		Ref.	
-Yes	5 (33.3%)	10 (66.7%)		1.36 (0.45-4.69)	
Extra-thyroidal extension			0.702[b]		140
-No	54 (40.6%)	79 (59.4%)		Ref.	
-Yes	2 (28.6%)	5 (71.4%)		1.63 (0.32-13.1)	
Intra-thyroidal extension			0.362[b]		140
-No	53 (41.4%)	75 (58.6%)		Ref.	
-Yes	3 (25.0%)	9 (75.0%)		2.04 (0.57-10.1)	
Presence of multicentricity			0.803[b]		140
-No	36 (41.4%)	51 (58.6%)		Ref.	
-Yes	20 (37.7%)	33 (62.3%)		1.16 (0.58-2.37)	
K-ras mutation			>0.999[b]		48
-Negative	15 (42.9%)	20 (57.1%)		Ref.	
-Positive	6 (46.2%)	7 (53.8%)		0.88 (0.24-3.33)	
N-ras mutation			>0.999[b]		41
-Negative	16 (43.2%)	21 (56.8%)		Ref.	
-Positive	2 (50.0%)	2 (50.0%)		0.77 (0.07-8.04)	
BRAF mutation			>0.999[b]		52
-Negative	18 (45.0%)	22 (55.0%)		Ref.	
-Positive	5 (41.7%)	7 (58.3%)		1.14 (0.30-4.56)	
White blood cell, median (min-max)	7.2 (4.1-11.5)	7.0 (4.2-12.6)	0.875[a]	1.05 (0.85-1.28)	139
-Neutrophil, median (min-max)	4.2 (2.2-7.6)	3.9 (1.6-10.0)	0.710[a]	0.96 (0.75-1.21)	139
-Lymphocyte, median (min-max)	2.2 (0.9-4.0)	2.4 (1.2-4.5)	0.085[a]	1.66 (0.99-2.79)	139
-Neutrophil / Lymphocyte Ratio (NLR), median (min-max)	1.8 (0.8-6.3)	1.6 (0.7-7.7)	0.067[a]	0.78 (0.57-1.07)	139

[a]Mann-Whitney U test, [b]Chi-square test, [c]Odds ratio (univariate logistic regression), *p<0.05

IC: Indeterminate cytology, **OR**: Odds ratio, **SD**: Standard deviation, **TSH**: Thyrotropin releasing hormone, **Anti-TPO**: Anti thyroid peroxidase antibody, **FNAB**: Fine-needle aspiration biyopsy

DISCUSSION

Thyroid nodules become more common with age, and the majority are found after the age of 40. Thyroid USG is accepted as the first-line imaging tool for thyroid nodule assessment. High-resolution linear probes allow for excellent identification of thyroid nodules by experienced operators. To assess thyroid nodules and their risk of malignancy, the radiologist should provide detailed information on the echogenicity, margin, shape and composition (cystic/solid) of the nodule, the presence of calcifications and the characteristics of the cervical lymph nodes[12]. In addition to these ultrasonographic findings, some other factors should be taken into account when deciding to perform FNAB include childhood irradiation, the presence of hereditary syndromes for thyroid cancer, a family history of thyroid cancer, the presence of a hard and/or fixed nodule, hoarseness, dyspnea, dysphagia, difficulty swallowing, and a rapidly growing nodule[13]. Ultrasonographic findings such as hypoechogenicity, infiltrative, irregular or spiculated margins, intranodular microcalcification and taller-than-wide shape are strongly associated with malignancy. In addition to nodule characteristics, the presence of suspicious lymph nodes should be noted in the USG report[14]. The malignancy risk of the existing nodule and the indication for FNAB are determined according to risk scoring systems based on these findings[7,15-17].

USG guided FNAB is the gold standard method to evaluate the suspected nodules[18]. It is a reliable and cost-effective procedure that is routinely used to diagnose the histopathologic features of thyroid nodule[19]. Currently, the likelihood of false negative results of FNAB is the most important problem in the preoperative evaluation of thyroid nodules. FNAB false negativity can be up to 13% for benign FNABs and up to 40% for indeterminate cytology FNABs[20]. In our series, the false negative rate was 38/179 (21.2%) for benign FNAB results and 21/49 (42.8%) for ND and AUS/FLUS FNAB results. Since we decided to selectively operate on nodules with increased radiologic and clinical suspicion of malignancy in addition to the FNAB result, false negative rates were higher in our cohort compared to the literature.

Some of the studies revealed that large nodules (>4 cm) have increased risk for malignancy[20], whereas others note the contrary[17] according to literature. Although the general approach in the literature is that FNAB false-negativity is high in nodules >4 cm[20-22], there are also publications reporting that false-negativity in USG-guided FNAB is independent of nodule size[23-25]. Carillo et al.[22], revealed that false negativity rates in nodules with benign FNAB results was 20% for >4.0 cm nodules and 5.1% for <4 cm nodules. Rosario et al.[26] evaluated >4.0 cm nodules with benign FNAB findings and reported a false negative rate of 3.6%. In another study conducted by Kuru et al.[23], reported that the false-negativity rate was 4.3% for >4 cm nodules and 1.3% for <4 cm nodules. They also revealed that the important point was not the size of nodule, but the size of thyroid carcinoma in the nodules. The size of the nodule was found to have no effect on false-negative FNAB results (p:0.313), in our study. However, in the analyzes performed in the IC subgroup, the median tumor size was found to be smaller in patients with false negative FNAB (30.0mm vs. 18.0mm, p:0.003). Bestepe et al.[19], determined that malignancy risk of nodules >4 cm was similar with the nodules <4 cm in size. The false-negativity rate for nodules between 1.0-3.9 cm in size was 2.2%, which was lower than nodules with size of <1 cm and >4 cm. Consistent with our study, Shrestha et al.[24] stated that the false-negativity rates of >4 cm and 1-4 cm nodules in size were similar (7.1% and 6.3%, respectively), on the other hand it was statistically higher (15.8%) in nodules <1 cm in size. Renshaw et al.[27] stated that the sensitivity of FNAB for 9 mm papillary carcinomas is 44.3%. Higher false-negative FNAB rates in small nodules can be explained by the technical difficulties and the sampling errors according to small size of the nodules.

The presence of micro/macro-calcification on preoperative USG had no statistically significant effect on the false negativity of FNAB in our study. Consistent with our cohort, Bestepe et al.[19], revealed that preoperative USG features had no effect to preoperatively detect the false negative outcomes of FNAB.

One of the parameters affecting the cytopathologic diagnosis of IC nodules is the presence of concurrent thyroiditis in extranodular thyroid tissue. According to the Bethesda reporting system, the potential of Hurthle cell hyperplasia may rise in clinical circumstances such as thyroiditis, and the detection of Hurthle cell-rich aspirate on FNAB may be appropriate to be classified as AUS/FLUS rather than Hurthle cell neoplasia. According to the limited

available data, the influence of Hashimoto's thyroiditis on the risk of cancer in Hurthle cell-rich aspirates is relatively small and does not cause particular concern[28,29]. Belfiore et al. stated that the clinician should be careful about false negativity of FNAB, especially in cases of cystic degeneration, the presence of Hurthle cells or chronic thyroiditis. It was aimed to determine and compare the relationship between serum Anti-TPO and NLR values, which may be an indicator and laboratory indicator of changes in thyroid tissue in the presence of thyroiditis during the preoperative period, and the presence of FNAB false negativity. In our cohort, the presence of thyroiditis had no effect on the false negativity of FNAB, but preoperative serum Anti-TPO elevation was higher in patients with false negative FNAB (Group-1) (60% vs. 40%, p:0.027). Analyses of the IC patient subgroup revealed that the presence of thyroiditis statistically significantly increased the false negativity of FNAB (IC/Group-1) (75% vs 25%, p:0.023)[30].

In recent years, there have been publications stating that NLR increases are detected in the presence of both thyroid cancer and thyroiditis. Ari et al.[31], revealed that the NLR was significantly higher in Hashimoto thyroiditis patients than in healthy controls. It was also higher in patients with papillary cancer, but the difference was not statistically significant. Consistent with these findings, NLR was statistically significantly higher in IC/Group-1 in our cohort, which was similar to thyroiditis statistics.

Mutation tests can be performed to make a surgical decision, especially for nodules in the IC group. Shrestha et al[32], revealed that following the identification of a mutation, the risk of malignancy was increased to 15-45% in AUS/FLUS and 53% in SFN. However we did not detected statistically significant difference between groups in terms of the results of mutation tests in our cohort and subgroup analysis.

When we compared the final pathologies of the patients, FNAB true-positivity was found to be statistically significantly higher in the presence of LVI/PNI, extra-thyroidal extension, and multi-centricity. In the IC subgroup, FNAB false-negative tumors were compared with true-positive tumors, FNAB true positivity was found to be higher in "non-encapsulated" tumors and in the presence of "classic variant". In the light of all these data, it can be revealed that the accuracy of FNAB increases in cases with highly aggressive tumor and classical variant.

The primary limitation of our study is its retrospective nature. In addition, selection bias regarding the inclusion of patients who underwent thyroidectomy cannot be excluded. Therefore, the accuracy of FNAB could not be calculated in patients who did not undergo thyroidectomy. In addition, the small number of patients who underwent mutation testing is another limitation of our study.

It will be possible to support the results of this study with prospective studies including patients who underwent thyroidectomy and were followed up without thyroidectomy in order to demonstrate the accuracy of FNAB in nodules developing on the basis of thyroiditis.

In conclusion, the diagnostic accuracy of FNAB increases in classical variant tumors and in tumors with aggressive pathological findings such as extra-thyroidal extension, perineural/lymphovascular invasion, non-encapsulation and multicentricity. However, the presence of thyroiditis increases the false negative rates of FNAB, especially in the IC patient group. Accordingly, high serum anti-TPO and NLR levels increase the false negativity of FNAB. Small tumor size is another factor for FNAB false negativity.

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