Ultrasonographic Characteristics of Thyroid Nodules in Predicting the Malignancy and the Role of the Cytopathologist

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ABSTRACT

Aim: It is very important to determine the correct nodules which should be evaluated with thyroid fine-needle aspiration biopsy (FNAB) and avoid unnecessary operations. In this study, we aimed to analyze the relationship and the compatibility between thyroid FNAB results with ultrasonographic features and the role of the pathologist who examines the specimens.

Material and Methods: 458 patients with nodules who underwent FNAB were included in the study. The relationships between the ultrasonographic features (echogenicity, shape, margin, vascularization and calcification) and biopsy results and the factor of the pathologist were evaluated retrospectively. Since there was no Bethesda VI (malignant) result, analyzes were made among 5 subgroups. FNAB procedures were considered as, inadequate or nondiagnostic, benign, atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), follicular neoplasia and suspicion of malignancy.

Results: Solid internal structure, irregular margin, hypoechogenicity, presence of microcalcification, nodules with taller than wide (AP> T) were found significantly different between the subgroups of Bethesda classification system (p<0.01, for all features). All suspicious features were found more common in the malignant suspicious nodules than the benign nodules. 63 of 211 preparations (29.8%), which were evaluated as Bethesda I and Bethesda III, were reported by a single pathologist, and statistically significant differences were found in comparison to the other pathologists (p<0.01).

Conclusion: Ultrasonography features that include malignancy risk correlate with cytopathology results. The incidence of thyroid nodules is gradually increasing and therefore, it is important to perform biopsies according to ultrasonographic risk stratification. Moreover, working with a thyroid specialist pathologist may minimize the indeterminate results.

Keywords: Thyroid nodule, Ultrasound imaging, Aspiration biopsy, Fine-needle

ÖZ

Amaç: Tiroid ince iğne aspirasyon biyopsisi ile değerlendirilmesi gereken doğru nodülleri belirlemek ve gereksiz işlemlerden kaçınmak çok önemlidir. Bu çalışmada tiroid ince iğne aspirasyon biyopsi sonuçları ile ultrasonografik özellikler arasındaki ilişki ve patoloğun rolünü incelemeyi amaçladık.

Gereç ve Yöntemler: Çalışmaya tiroid ince iğne aspirasyon biyopsisi yapılan 458 hasta dahil edildi. Ultrasonografik özellikler (ekojenite, şekil, vaskularizasyon, sınır ve kalsifikasyon) ve biyopsi sonuçları ile patolojik faktörler arasındaki ilişki retrospektif olarak değerlendirildi. Bethesda VI (malign) sonucu olmadığı için 5 alt grup arasında analizler yapıldı. İİAB işlemler patoloji sonuçları açısından yetersiz, benign, önemi belirsiz atipi/öneni belirsiz folliküler lezyon, folliküler neoplazi ve malignite şüphesi olarak değerlendirilmiştir.
INTRODUCTION

Although most thyroid nodules show benign features, thyroid gland carcinomas are the most common malignancies in the endocrine system. According to the United States cancer database system (SEER), the incidence of papillary thyroid carcinoma was 4.8 per 100000 in 1975 and 14.9 per 100000 in 2012. Early diagnosis is important because thyroid malignancies mostly have a slow progression, and survival rates are high with appropriate treatment. For this reason, all nodules should be examined for ultrasonographic features and evaluated histopathologically in the case of suspected ultrasonography (US) findings. None of the ultrasonographic features predicts or excludes malignancy by 100% percent. However, the presence of 2 or more suspicious sonographic findings increases the risk of malignancy (1). The most convenient and reliable method that can reveal the histopathological feature of the nodule is thyroid fine-needle aspiration biopsy (FNAB). Performance of biopsy by an experienced clinician with US guidance and evaluation of samples by an experienced cytopathologist increase the diagnostic value. Retrospective studies have shown that, in comparison to palpation, both the false negativity and the non-diagnostic rates are much lower in FNAB performed with US guidance (2).

In this study, we aimed to retrospectively analyze the relationship and the compatibility between thyroid FNAB results and ultrasonographic features with the recommendation of the guidelines and the relationship between cytological results and role of pathologists in a single center.

MATERIALS and METHODS

Four hundred and fifty-eight patients who underwent FNAB on their thyroid nodules between June 2015 and October 2017 at the Endocrinology Clinic of Samsun Research and Training Hospital were included in the study. This study was conducted in accordance with the declaration of Helsinki, and the protocol for this retrospective analysis was approved by the Local Ethics Committee (Ondokuz Mayis University, Clinical Research Ethics Committee, 09.07.2020, 2020/462 decision number). The data of the patients were evaluated retrospectively. In all patients, nodule evaluation was performed with the high-resolution probe (13-5 MHz, linear) of the same ultrasonography device (Siemens Antares). Based on ultrasonography; solid internal structures, irregular borders, hypoechochogenicity, presence of microcalcification and nodules with taller than wide (AP>T) were accepted as suspicious features (3).

Three dimensions were measured, and the largest diameter in millimeters was taken into consideration. The echogenicity of the nodules were compared to the surrounding thyroid parenchyma and classified as isoechoic, hypoechoic, hyperechoic and mixed. Mixed echoic nodules were evaluated based on their internal solid components. The echogenicity of the solid portion was categorized as hyperechoic (for nodules showing hyperechochogenicity compared to the normal thyroid), isoechoic (for nodules showing isoechochogenicity compared to the normal thyroid), or hypoechoic (for nodules showing hypoechochogenicity compared to the normal thyroid). The composition of the nodules was classified as solid, cystic, predominantly solid (<50% cystic components), predominantly cystic lesions (≤50% solid components), or spongiform. The margin was classified as regular or irregular, while calcification was classified as microcalcification, macrocalcification or no calcification. Vascularization was classified as absent vascularity (type 1), perinodular vascularity greater than intranodular vascularity (type 2) or intranodular vascularity greater than peripheral vascularity (type 3). The feature of AP>T was indicated as present or absent.

Fine-needle aspiration biopsies were performed by applying vacuum with a 22-gauge needle. Air-dried specimens were taken for cytopathological examination. The specimens were randomly examined by 7 different pathologists (1st Pathologist, 2nd Pathologist, 3rd Pathologist, 4th Pathologist, 5th Pathologist, 6th Pathologist, 7th Pathologist) according to their working schedule. The results were reported in six different categories according to the Bethesda classification system (4). Bethesda I: nondiagnostic or inadequate, Bethesda II: benign, Bethesda III: atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), Bethesda IV: follicular neoplasm or suspicious for follicular neoplasm, Bethesda V: suspicious for malignancy, Bethesda VI: malignant.
Statistical Analysis

The Number Cruncher Statistical System (NCSS) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the data of the study, descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Rate, Minimum, Maximum) were used, and the distribution of the data was evaluated with Shapiro-Wilk Test. Kruskal-Wallis Test was used for comparison of three or more groups that did not show normal distribution of the quantitative data, ANOVA was used for comparison of three or more groups with normal distribution, and post hoc tests were performed to determine the difference. The qualitative data were analyzed with the Chi-square test. Qualitative data were analyzed with the Chi-square test. Fisher-Exact analysis was used for two groups and Monte-Carlo analysis for multiple groups. Significance was evaluated at $p <0.01$ and $p <0.05$ levels.

RESULTS

A total of 458 people, 396 women (86.5%) and 62 men (13.5%), were included in the study. The mean age of the patients was 52.07 ± 12.3 years. The mean nodule size was 19.48 ± 7.46 mm. 225 (49.1%) nodules were located on the right lobe, 211 (46.1%) were located on the left lobe, and 22 (4.8%) were located on the isthmus. 202 nodules were isoechoic (44.1%), 129 nodules were hypoechoic (28.2%), 4 nodules were hyperechoic (0.9%), and 123 nodules were mixed (26.8%). 321 nodules were solid (70.1%), 13 nodules were cystic (2.8%), 118 nodules were mixed (predominantly cystic and predominantly solid) (25.8%), and 6 nodules were detected in a spongiform (1.3%) structure. 442 nodules had regular margins (96.5%), and 16 nodules had irregular margins (3.5%). There were microcalcifications (5%) in 23 nodules and macrocalcifications (4.4%) in 20 nodules. Calcification was not observed in 415 nodules (90.6%). 164 (35.8%) had Type 1, 290 (63.3%) had Type 2 and 4 (0.9%) had Type 3 vascularization patterns. The results are shown in Table 1.

When the FNAB procedures were evaluated in terms of the pathology results, 80 (17.5%) were inadequate, 189 (41.3%) were benign, 131 (28.6%) were AUS/FLUS, 42 (9.2%) were follicular neoplasia, and 16 (3.5%) were found to be suspected of malignancy. Since there was no Bethesda VI (malignant) result in the FNAB results, analyses were conducted among 5 subgroups. There was no statistically significant difference between the groups in terms of age and sex ($p = 0.136$, $p = 0.145$). There was no statistically significant difference between the groups in terms of the nodule sizes and localizations ($p=0.072$, $p=0.085$). While irregular margins were detected by ultrasonography in 10 of 58 nodules with AUS/FLUS and suspected malignancy, none of the benign nodules had irregular margins ($p<0.001$). 75% of the nodules were hypoechoic in the group with suspected malignancy, and 19% were hypoechoic in the benign group ($p = 0.001$; $p <0.01$). When the Bethesda subgroups were compared in terms of nodule calcification, a statistically significant difference was found between the groups ($p <0.01$). Microcalcification was detected by 43.8% in the group with suspected malignancy and by 1.6% in the benign group. While none of the benign nodules had AP> T, 12.5% AP> T was found in the malignancy-suspected group ($p = 0.001$; $p <0.01$). In terms of vascularization, 75% Type 2 and 12.5% Type 3 vascularization results were found in the nodules with suspicion of malignancy, while none of the benign nodules had Type 3 vascularization. The results are shown in Table 2.

A statistically significant difference was found in terms of the results and the pathologists. Bethesda I and Bethesda III that required repeated biopsy were evaluated in terms of the pathologists who examined the results. 32 (40%) of 80 preparations were reported as Bethesda I by the 1st Pathologist, 13 (16.3%) were reported by the 2nd Pathologist, 11 (13.8%) were reported by the 3rd Pathologist, 13 (16.3%) were reported by the 4th Pathologist, 1 (1.3%) was reported by the 5th Pathologist, 8 (10%) were reported by the 6th Pathologist.

Table 1. Characteristics of the patients and thyroid nodules

| Characteristics | Results |
|-----------------|---------|
| Age             | Mean±SD | 52.07±12.30 |
|                 | Min-Max (Median) | 17-88 (53.0) |
| Nodule Size     | Mean±SD | 19.48±7.46 |
|                 | Min-Max (Median) | 1-45 (18.0) |
| Gender          |         | Female 396 (86.5) |
|                 |         | Male 62 (13.5) |
| Echogenicity    |         | Isoechoic 201 (44) |
|                 |         | Hypoechoic 129 (28.1) |
|                 |         | Hyperechoic 4 (0.9) |
|                 |         | Mixed 124 (27) |
| Margin          |         | Regular 442 (96.5) |
|                 |         | Irregular 16 (3.5) |
| Calcification   |         | Micro 23 (5) |
|                 |         | Macro 20 (4.4) |
|                 |         | No calcification 415 (90.6) |
| Vascularization |         | Type 1 164 (35.8) |
|                 |         | Type 2 290 (63.3) |
|                 |         | Type 3 4 (0.9) |
|                 |         | AP>T Present 4 (0.9) |
|                 |         | Absent 454 (99.1) |

Table 2. Results of the statistical analysis

| Statistic | Mean±SD | Min-Max (Median) |
|-----------|---------|------------------|
| ANOVA     |         |                  |
| Chi-Square|         |                  |
| Fisher-Exact|      |                  |
| Monte-Carlo|       |                  |
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Pathologist and, 2 (2.5%) were reported by the 7th Pathologist. Among the 131 preparations reported as Bethesda III, 31 (23.7%) were reported by the 1st Pathologist, 11 (8.4%) were reported by the 2nd Pathologist, 15 (11.5%) were reported by the 3rd Pathologist, 18 (13.7%) were reported by the 4th Pathologist, 15 (11.5%) were reported by the 5th Pathologist, 31 (23.7%) were reported by the 6th Pathologist, and 10 (7.6%) were reported by the 7th Pathologist. The results are shown in Table 3.

**DISCUSSION**

In this study, when the high-risk features of US and cytopathology results were compared, a statistically significant relationship was found between hypoechogenicity, irregular margin, microcalcification, and AP> T and malignancy in accordance with the literature. Additionally, a statistically significant relationship was found between the reports which required repeated biopsy (Bethesda I and Bethesda III) and the pathologists who examined the preparations.

Fine-needle aspiration biopsy is the most reliable and inexpensive method to evaluate thyroid nodules. In ultrasonography, hypoechogenicity, intranodular vascularization, irregular margin, microcalcification and AP> T are risk factors for malignancy. The solid component is the most sensitive sonographic finding for malignancy. However, its specificity is 52-56% (5). The risk of malignancy increases with a decrease in echogenicity. The marked hypoechoic nodule has 92% specificity and 26-41% sensitivity for malignancy (6,7). In our study, while 75% of the nodules with suspected malignancy were hypoechoic, this rate was found to be 19% in the benign nodules.

**Table 2**: Comparison of Bethesda system subgroups in terms of nodule ultrasonographic characteristics

| Gender | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|--------|------------|-------------|---------------|-------------|------------|-------|
| Female | 63 (15.9)  | 164 (41.4)  | 117 (29.5)    | 39 (9.8)    | 13 (3.3)   | 0.145 |
| Male   | 17 (27.4)  | 25 (40.3)   | 14 (2.6)      | 3 (4.8)     | 3 (4.8)    |       |

| Echogenicity | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|--------------|------------|-------------|---------------|-------------|------------|-------|
| Iso          | 23 (1.4)   | 109 (54.2)  | 49 (24.4)     | 17 (8.5)    | 3 (1.5)    | 0.042*|
| Hypo         | 32 (24.8)  | 36 (27.9)   | 37 (28.7)     | 12 (9.3)    | 12 (9.3)   |       |
| Hyper        | 3 (75)     | 1 (25)      | 0 (0)         | 0 (0)       | 0 (0)      |       |
| Mixed        | 22 (17.7)  | 43 (34.7)   | 45 (36.3)     | 13 (10.5)   | 1 (0.8)    |       |

| Margin       | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|--------------|------------|-------------|---------------|-------------|------------|-------|
| Regular      | 78 (17.6)  | 189 (42.8)  | 127 (28.7)    | 39 (8.8)    | 9 (2)      | 0.001**|
| Irregular    | 2 (12.5)   | 0 (0)       | 4 (25)        | 3 (18.8)    | 7 (43.8)   |       |

| Calcification | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|---------------|------------|-------------|---------------|-------------|------------|-------|
| Micro         | 3 (13)     | 3 (13)      | 6 (26.1)      | 4 (17.4)    | 7 (30.4)   | 0.001**|
| Macro         | 7 (35)     | 11 (55)     | 2 (10)        | 0 (0)       | 0 (0)      |       |

| Vascularization | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|-----------------|------------|-------------|---------------|-------------|------------|-------|
| Type-1          | 36 (22)    | 87 (53)     | 33 (20.1)     | 6 (3.7)     | 2 (1.2)    | 0.001**|
| Type-2          | 43 (14.8)  | 102 (35.2)  | 97 (33.4)     | 36 (12.4)   | 12 (4.1)   |       |
| Type-3          | 1 (25)     | 0 (0)       | 1 (25)        | 0 (0)       | 2 (50)     | 0.001**|

| AP>TR          | Bethesda I | Bethesda II | Bethesda III | Bethesda IV | Bethesda V | p     |
|----------------|------------|-------------|---------------|-------------|------------|-------|
| Present        | 1 (25)     | 0 (0)       | 1 (25)        | 0 (0)       | 2 (50)     | 0.001**|
| Absent         | 79 (17.4)  | 189 (41.6)  | 130 (28.6)    | 42 (9.3)    | 14 (3.1)   |       |

Chi-Square Test **p<0.01, * p<0.05

**Table 3**: Comparison of cytology results in terms of pathologists who evaluate the specimens

| Bethesda | 1st Pathologist | 2nd Pathologist | 3rd Pathologist | 4th Pathologist | 5th Pathologist | 6th Pathologist | 7th Pathologist |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Bethesda I | 32 (40)         | 13 (16.3)       | 11 (13.8)       | 13 (16.3)       | 1 (1.3)         | 8 (10)          | 2 (2.5)         | 0.001 |
| Bethesda II | 3 (1.6)         | 35 (18.5)       | 27 (14.3)       | 2 (1.1)         | 54 (28.6)       | 12 (6.3)        | 56 (29.6)       |
| Bethesda III | 31 (23.7)       | 11 (8.4)        | 15 (11.5)       | 18 (13.7)       | 15 (11.5)       | 31 (23.7)       | 10 (7.6)        |
| Bethesda IV | 0 (0)           | 2 (4.8)         | 2 (4.8)         | 9 (21.4)        | 5 (11.9)        | 14 (33.3)       | 10 (23.8)       |
| Bethesda V  | 0 (0)           | 6 (37.5)        | 3 (18.8)        | 0 (0)           | 2 (12.5)        | 2 (12.5)        | 3 (18.8)        |
In comparison to a non-calcified solid nodule, the risk of cancer is 3 times higher in the presence of microcalcification. In predicting malignancy, microcalcification's specificity is 93%, its positive predictive value is 70%, but its sensitivity is 36% (8). Macrocalcification is less specific for malignancy and is often observed in benign multiple nodules. However, its presence in a solitary nodule may be a finding in favor of malignancy by 75%. In our study, microcalcification was detected in 43.8% of the nodules with suspected malignancy and 1.6% of the nodules with benign results. While 5.8% of the nodules with benign results had macrocalcification, none of the patients with suspected malignancy had macrocalcification. Although the margin interpretations of nodules show subjective differences, the specificity of an irregular margin in predicting malignancy is 83-91.8%, and its sensitivity is 48.3-55% (6,7). The results in this study were also compatible with the literature. While nodules that have a dominantly peripheral vascularization are benign, nodules showing a chaotic intranodular bleeding pattern have a potential to become malignant. The specificity of internal hypervascularization in predicting malignancy is 81%, and its sensitivity is 74% (1). In our study, 75% Type 2 and 12.5% Type 3 vascularization patterns were found in the nodules with suspicion of malignancy, while none of the benign nodules had Type 3 vascularization.

Although fine-needle aspiration biopsy is an important technique in evaluation of nodules, the adequacy of the specimen, sampling technique, the experience and skill of the pathologist may cause differences in evaluations. In the cytology of thyroid lesions, diagnostic variations and disagreements between pathologists are encountered, especially for the diagnosis of follicular lesions and follicular neoplasms. In a study in which preparations diagnosed as follicular lesions were examined by 4 different pathologists, a moderate to significant evaluation difference was detected among the pathologists (9). Seok et al. found that, with examination of thyroid FNAB by thyroid specialist cytopathologists, more accurate results could be obtained in a shorter time (10). In another study, it was found that, if the preparations that were reported as AUS / FLUS were reevaluated with the joint examination of at least five pathologists, there was an optimal level of inter-observer consistency, less indetermination and excellent cytohistological compatibility (11). In a review, it was shown that, if thyroid FNAB samples were evaluated by a second cytopathologist, the diagnostic accuracy increased, and clinical management was potentially changed (12). It has also been described as an important diagnostic method for precise recognition and improved accuracy for biopsies, which were originally defined as "uncertain". In our study, 63 of 211 preparations (29.8%), which were evaluated as insufficient and indeterminate, were reported by a single pathologist, and statistically significant differences were found in comparison to the other pathologists.

There were some strengths and limitations of our study. All thyroid US and FNAB procedures were performed by the same clinician. It eliminated clinician-related differences. Furthermore, all FNAB procedures were performed with US guidance. Although we aimed to compare the diagnostic accuracy of different pathologists, histopathological diagnosis could not be used as the reference method because of missing data of operation materials. This was because most of the operations were performed out of our hospital. Since it was a retrospective study, the same specimens could not be evaluated by each pathologist. Additionally, the number of the samples evaluated by each pathologist was small.

In conclusion, the incidence of thyroid nodules is gradually increasing, and therefore, it is important to perform biopsies based on ultrasonographic risk stratification. Working with specific cytopathologists who will be able to evaluate thyroid biopsies will be an appropriate strategy to prevent unnecessary repeated biopsies and operations.

**REFERENCES**

1. Papini E, Guglielmi R, Bianchini A, Crescenzi A, Taccogna S, Nardi F, Panunzi C, Rinaldi R, Toscano V, Pacella CM. Risk of malignancy in nonpalpable thyroid nodules: Predictive value of US and color- Doppler features. J Clin Endocrinol Metab 2002;87:1941-1946.

2. Danese D, Sciacchitano S, Farsetti A, Andreoli M, Pontecorvi A. Diagnostic accuracy of conventional versus sonography-guided fine-needle aspiration biopsy of thyroid nodules. Thyroid 1998;8:15-21.

3. Russ G, Bonnema SJ, Erdogan MF, Durante C, Ngu R, Leenhardt L. European thyroid association guidelines for ultrasound malignancy risk stratification of thyroid nodules in adults: The EU-TIRADS. Eur Thyroid J 2017;6:225-237.
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4. Cibas ES, Ali SZ. The 2017 Bethesda system for reporting thyroid cytopathology. Thyroid 2017;27:1341-1346.
5. Frates MC, Benson CB. Society of Radiologists in Ultrasound. Management of thyroid nodules detected at US: Society of Radiologists in US consensus conference statement. Radiology 2005;237:794-800.
6. Moon WJ, Jung SL, Lee JH, Na DG, Baek JH, Lee YH, Kim J, Kim HS, Byun JS, Lee DH, Thyroid Study Group, Korean Society of Neuro- and Head and Neck Radiology. Thyroid nodules: Re-evaluation with ultrasound. J Clin Ultrasound 1995;23:179-184.
7. Clary KM, Condel JL, Liu Y, Johnson DR, Grzybick DMi, Raab SS. Variability in the fine needle aspiration biopsy diagnosis of follicular lesions of the thyroid gland. Acta Cytol 2005;49:378-382.
8. Seok JY, An J, Cho HY. Improvement of diagnostic performance of pathologists by reducing the number of pathologists responsible for thyroid fine needle aspiration cytology: An institutional experience. Diagn Cytopathol 2018;46:561-567.
9. Jing X, Knoepp SM, Roh MH, Hookim K, Placido J, Davenport R, Rasche R, Michael CW. Group consensus review minimizes the diagnosis of “follicular lesion of undetermined significance” and improves cytohistologic concordance. Diagn Cytopathol 2012;40:1037-1042.
10. Gerhard R, Boerner SL. The value of second opinion in thyroid cytology: A review. Cancer Cytopathology 2014;122:611-619.