Original Article

Thyroid FNAC containing hürthle cells and hürthle-like cells: A study of 128 cases

ABSTRACT

Aim: It is a diagnostic challenge to differentiate benign and malignant cytology in the presence of Hürthle cells. In our previous study, it was determined that in fine needle aspirations (FNA), the malignancy outcome of the Hürthle cells containing group tend to be papillary thyroid carcinoma (PTC) in a higher percentage. The most common misinterpretation is caused by PTC cells with large cytoplasm-like Hürthle cells. The aim of this study is to predict histologic outcome of the nodules, which have Hürthle cells in FNA according to cytological, clinical features, and *BRAF*^{V600E} mutation status.

Materials and Methods: Detailed cytological features of 128 cases were compared with histopathological diagnosis. The analysis of *BRAF*^{V600E} mutation of the PTC cases were performed by real-time polymerase chain reaction.

Results: The neoplastic outcome was increased statistically significantly with younger age (P = 0.020), increase in cellular dyshesion (P = 0.016), presence of nuclear budding (P = 0.046), and granular chromatin (P = 0.003). Nuclear budding (P = 0.014), granular chromatin (P = 0.012), and hypoechoic nodules in ultrasonography (P = 0.011) were significant independent factors for the increase in the malignancy risk. Increased lymphocytes (P = 0.015) and colloid were related to non-neoplastic outcome. According to the surgical outcome, more than half of the malign cases were PTC (74%). *BRAF*^{V600E} mutation was detected in 27.8% of the PTC cases.

Conclusion: PTC cases containing Hürthle cell-like cells may lead to diagnostic errors. Nuclear budding and granular chromatin of Hürthle cells are significant, remarkable findings to predict the outcome of neoplasm and malignancy.

Key words: Hürthle cell; fine needle aspiration; thyroid

Introduction

Hürthle cells are large polygonal cells with eosinophilic granular cytoplasm, which show the accumulation of mitochondria.[1] Askanazy first described Hürthle cells in 1898. Hürthle cells are derived from thyroid follicular cells.[2] It is a diagnostic challenge for cytopathologists to

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differentiate and interpret benign and malignant nodules of thyroid in the presence of Hürthle and/or Hürthle-like cells. In our previous study, we observed that the rates of atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) and follicular neoplasm (FN)/Suspicious for a follicular neoplasm (SFN) categories were higher in the presence of Hürthle cells (9.6% versus 4% and 3.6% versus 0.7%, respectively).[3] After surgery, neoplastic and malignant outcomes were significantly higher in Hürthle cells containing cases in fine needle aspirations (FNA) (27.3% versus 14.9% and 21.1% versus 11.7%, respectively).[3] In 74% of the cases with Hürthle cells in aspiration and with malignant outcome, final diagnosis was papillary thyroid carcinoma (PTC).[3] It was suggested that the high incidence of PTC is mostly related to misinterpretation of PTC cells as Hürthle cells in cytology. Another probable reason was using air-dried slides with May Grunewald Giemsa (MGG) staining. The aim of this study is to predict the histologic outcome of the nodules, which have Hürthle cells in FNA according to cytological, clinical features, and *BRAF*^{V600E} mutation status and whether it is possible to avoid misinterpretation of large cells in PTC as Hürthle cells.

Materials and Methods

It was decided to analyze the detailed cytological features of Hürthle-like cells in MGG slides of the cases with follow up information specially to discover specific features of the PTC outcome. Detailed clinical and radiological features that may aid in reaching correct diagnosis were also collected. In addition, *BRAF* mutation status was studied from the PTC cases to determine the mutation incidence in order to help the cytological diagnosis in the presence of Hürthle-like cells.

A total of 895 FNAs that contain Hürthle cells in cytopathological reports were detected, and 128 of the cases had surgical outcome. This study was a retrospective analysis of these 128 cases. Institutional Review Board approval was obtained for the retrospective analysis of these 128 cases for the study. All cases were retrieved from the archives of the Department of Pathology. Informed consent was implied because of the retrospective design of this study.

Thyroid nodules had been described by ultrasonography and compared with macroscopic mapping and description in thyroidectomies. Papillary thyroid microcarcinomas were not excluded from the study. Aspirations were performed under ultrasonography guidance. Partial or complete thyroidectomies were sampled by mapping with detailed descriptions of the localization of nodules. Cytological features were compared with their corresponding thyroid nodules. The conventional smears were air dried and stained by the MGG method. The FNA samples were re-evaluated according to the recommendation of 2007 NCI Thyroid FNA State of the Science Conference.[4] A pathologist, who was blinded to the corresponding surgical outcome, reviewed the thyroid FNA of these 128 cases. A senior pathologist also reviewed doubtful cases. Table 1 shows the Bethesda distribution and related surgical outcome of 128 cases. A detailed cytological analysis was done subsequently. Background features (such as lymphocytic reaction and presence and amount of colloid) and nuclear and cytoplasmic features of cells were evaluated. Cytological features which were scored according to quantity are listed in Table 2.

Presence or absence of the following features were assessed in the slides: Nuclear budding in Hürthle cells [Figure 1a], macronucleoli, small and large cell dysplasia, transgressing vessels among Hürthle cells [Figure 1b], capillaries in the

Table 1: The Bethesda classification and surgical outcomes of 128 cases

	Total (cytology)	Benign	AUS/FLUS	FN/SFN	SFM	Malignant
Total *n* (Surgical outcome)	128 (100%)	86 (67.1%)	27 (21%)	11 (8.5%)	3 (2.3%)	1 (0.7%)
Multinodular goitre	71	57	11	3	0	0
Thyroiditis	22	16	6	0	0	0
Hürthle cell adenoma	1	0	0	1	0	0
Follicular adenoma	7	5	2	0	0	0
Hürthle cell carcinoma	3	0	0	3	0	0
Papillary carcinoma	17	5	7	2	2	1
Papillary micro carcinoma	3	1	0	1	1	0
Follicular carcinoma	3	2	1	0	0	0
WDTC	1	0	0	1	0	0
Total neoplastic *n* (%)	35 (27.3%)	13 (15.1%)	10 (37%)	8 (72.7%)	3 (100%)	1 (100%)
Total malignant *n* (%)	27 (21.1%)	8 (9.3%)	8 (29.6%)	7 (63.6%)	3 (100%)	1 (100%)
TBSRTC malignancy rates		0-3%	5-15%	15-30%	60-75%	97-99%

*AUS/FLUS = Atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN = Follicular neoplasm/suspicious for follicular neoplasm; SFM = Suspicious for malignancy; WDTC = well differentiated thyroid carcinoma; TBSRTC = The Bethesda system for reporting thyroid cytopathology

Table 2: Cytological analysis of the cases

Score	0	1	2
Cellularity	Low	Moderate	High
Colloid	Absent, scanty	Moderate, extensive	
Macrophages	Absent, rare	Moderate, extensive	
Lymphocytes	Absent	Rare, moderate	Extensive
Hürthle Cell Ratio	<10%	10-90%	>90%
Nuclear Enlargement	Absent	<10%	>10-50%
Cellular Dyshesion	Absent	<10-50%	>10-50%

background, binuclear Hürthle cells [Figure 1c], papillary structures, squamoid cytoplasm, granular chromatin [Figure 1c], gummy colloid, irregular membrane, oval nuclei, epitheloid giant cell, monolayer sheets, septate vacuoles, pseudoinclusion, nucleolus location, psammoma bodies, and nuclear grooves.

The descriptions from literature were used for the criteria. Nuclear budding was described as the nuclear membrane irregularity in the form of budding.[5] Budding of the nucleus is a result of the response to DNA destruction. Actual function of nuclear budding is the removal of amplified DNA.[6]

Nuclear diameter greater than two times the size of a red blood cell was defined as nuclear enlargement.[1] Small cell dysplasia was described as the cells having a diameter of less than twice the nuclear diameter. Large cell dysplasia was defined as cells demonstrating at least twice the variation in the nuclear diameter, often with prominent nucleoli and irregular nuclear outlines. Cellular dyshesion implied numerous single cells that were barely cohesive with each.[7] Transgressing blood vessels were described as capillaries passing through groups of Hürthle cells.[8]

Mutational analysis of *BRAF* codon 600 (600 GTG >GAG-1799T >A, *V600E*) was included for PTC cases. Paraffin blocks were available from 20 of the 15 PTC and three papillary thyroid microcarcinomas (micro-PTC) cases. A pathologist reviewed each case and chose the specimens with more than 90% of tumor cells. Genomic DNA was extracted from the cases, of which 10-μm sections of formalin-fixed and paraffin-embedded (FFPE) material by using conventional xylene/ethanol treatment, overnight incubation with proteinase K, and subsequent DNA purification utilizing the Pure Link Genomic DNA Mini Kit (USA). DNA concentration was measured with the Nano drop device (Thermo Scientific, Wilmington, DE). The standard polymerase chain reaction (PCR) protocol was performed with Bio Systems Step One Plus Real Time PCR (Fostercity, CA USA). Sequences were screened for *BRAF* codon 600 (600 GTG >GAG-1799T >A, V600E) with Entrogen BRAF mutation Analysis Kit (Tarxana, CA USA).

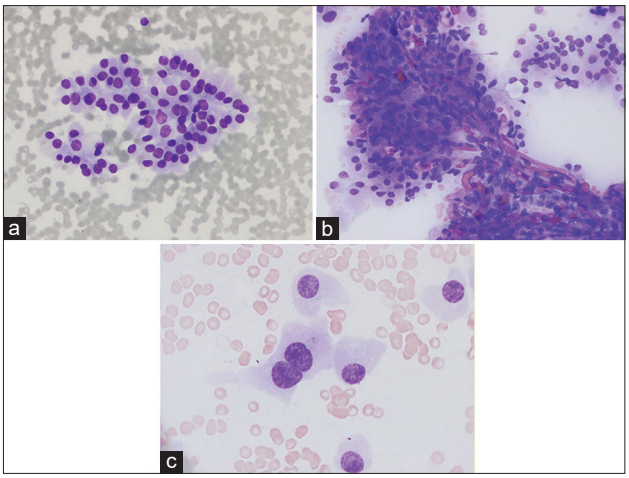

Figure 1: (a) Nuclear budding is seen in the center of Hürtle cell group (MGG (MGG stain, x200), ×400); (b) Vessels are passing through Hürthle cell group, also named as transgressing vessels (MGG (MGG stain, x100), ×400); (c) Binuclear Hürthle cells. Granular chromatin is seen in the nuclei (MGG stain, ×400)

Chi-square test and likelihood ratio were used to analyze the distribution of cytological diagnostic groups and neoplastic rates according to the presence of Hürthle cells. A value of $P < 0.05$ was accepted as statistically significant. Multivariate analyses using backward stepwise logistic regression models were also performed to determine independent predictive factors.

Results

The study was a retrospective analysis of 128 cases of thyroid cytology reports containing Hürthle (oncocytic) cells in the description with available follow-up histology. One hundred and eleven patients were females and 17 were males. Distribution of cytological diagnosis and histopathologic outcome were as described in Table 1.[3] In thyroidectomies, the distribution of the variants of PTC and micro-PTC were as follows; 7/18 (38.88%) follicular, 6/18 (33.33%) classic, 2/18 (11.11%) solid trabecular, and oncocytic 3/18 (16.66%9). The cytological diagnosis of the PTC cases according to the Bethesda system were 5/18 (27.7%) benign, 7/18 (38.8%) AUS/FLUS, 3/18 (16.6%) SFN, 2/18 (11.1%) SFM, and 1/18 (5.5%) malignant.

Binuclear cells ($P = 0.014$), small cell dysplasia ($P = 0.001$), large cell dysplasia ($P = 0.038$), nuclear enlargement ($P = 0.017$), nuclear budding ($P = 0.001$), cellular dyshesions ($P = 0.00$), increased Hürthle cell ratio ($P = 0.004$), papillary structures ($P < 0.001$), squamoid cytoplasm ($P < 0.001$), granular chromatin ($P < 0.001$), gummy colloid ($P = 0.019$), irregular membrane ($P < 0.001$) and oval nuclei ($P = 0.002$) were related to both neoplastic and malignant outcomes in univariate analyses. Transgressing blood vessels were not related to neoplastic or malignant outcome ($P = 0.070$).

Multivariate analyses were done to compare non-neoplastic and neoplastic outcomes [Hürthle cell carcinoma (HCC), Hürthle cell adenoma (HA), Follicular carcinoma (FC), Follicular adenoma (FA), (PTC)]. It was found that younger age ($P = 0.020$), increase in cellular dyshesions ($P = 0.016$), presence of nuclear budding ($P = 0.046$), and granular chromatin ($P = 0.03$) increased the neoplastic outcome significantly [Table 3].

On the other hand, the multivariate analysis for comparing benign and malignant outcomes (HCC, FC, PTC) revealed that the nuclear budding ($P = 0.014$), granular chromatin ($P = 0.012$), and hypoechoic nodules in ultrasonography ($P = 0.011$) were the significant independent factors for increased malignancy risk [Table 4]. Increased lymphocytes ($P = 0.015$) and colloid ($P = 0.018$) were related to non-neoplastic outcome.

There were 15 well-differentiated follicular tumors [(HA, FA, HCC, FC, well-differentiated thyroid carcinoma (WDTC)] and 20 PTC (including microcarcinomas) in our study. Small cell dysplasia ($P < 0.001$), presences of papilla formation ($P < 0.001$), squamoid cytoplasm ($P < 0.001$), granular chromatin ($P < 0.001$), irregular, nuclear membrane ($P < 0.001$) were more common in PTC. According to multivariate analyses, the presence of binuclear cells ($P = 0.022$), granular chromatin ($P = 0.03$), presence of papilla formation ($P = 0.002$) and increase in Hürthle cells ($P = 0.047$) were the significant independent factors for increased PTC risk [Table 5].

From paraffin slides of 15 papillary carcinoma and 3 papillary microcarcinoma cases, $BRAF^{V600E}$ mutation was detected in 5/18 (27.8%) specimens. The distribution of the mutations was; 2 classical variants, 1 classical variant papillary microcarcinoma, 1 follicular variant, 1 solid trabecular variant. $BRAF^{V600E}$ mutation was not detected in 13/18 (72.2%) specimens, which were 1 classical variant, 6 follicular variants, 1 classical variant papillary micro carcinoma, 1 solid trabecular variant papillary microcarcinoma, and 4 oncocytic variant.

Table 3: Variables of neoplastic outcome

Variable	Categories	*P* value	Odds ratio	95% CI
Nuclear Budding	Present	0.046	3.908	1.025-14902
Granular Chromatin	Present	0.003	5.815	1837-18400
Age	Increased	0.020	0.948	0.906-0.992
Cellular Dyshesion	10-50%	0.046	4.117	1.023-16.566
	>50%	0.016	12.752	1.611-100.941

Table 4: Comparison of independent factors for malignant outcome of tumors

Variables	Categories	*P* value	Odds ratio	95% CI
Granular Chromatin	Present	0.012	5.306	1.431-17.716
Nuclear Budding	Present	0.014	6.229	1.456-26.647
USG Hypoechoic	Present	0.011	7.370	1.569-34.627

Table 5: Significant independent factors for increased papillary thyroid carcinoma risk

Variables	Categories	*P* value	Odds ratio	95% CI
Binuclear Cells	Present	0.002	12319	1.4-105.95
Increase of Hürthle Cells	Present	0.047	12010	1.0-139.2
Presence of Papilla Formation	Present	0.002	199299	7.4-5327.7
Granular Chromatin	Present	0.031	6.626	1.2-36.8

Discussion

In our previous study, it was found that the malignancy outcome of the group containing Hürthle cells tend to be PTC in a higher percentage.[3] It was thought that the misinterpretation of PTC cells with large cytoplasm showing Hürthle-like features as Hürthle cells were the most common reason of this. This study was designed to find out the differentiation of these PTC-related Hürthle-like cells from Hürthle cells according to cytological, clinical features, and *BRAF* mutation status.

It was found that detailed cytomorphological examinations and clinical and radiological features were useful to predict surgical outcome with Hürthle cell lesions.

Prominent cellular dyshesion was a significant parameter in predicting neoplastic process in our study. Rozkos *et al*. studied E-cadherin expression in the resection specimens of the cases with cellular dishesion in aspirations. They demonstrated that follicular carcinoma had loss in cell cohesion compared to follicular adenoma.[9] Mitselou *et al*. also showed that E-cadherin expression was decreasing from benign to malignant lesions in thyroid pathologies.[10]

Nuclear budding was the most interesting criteria. Micronuclei and nuclear bud formation are considered to be biomarkers of genotoxic effects and chromosomal instability.[11] Dutra *et al*. demonstrated chromatin content in nuclear buds but they could not show a specific chromosome to form buds.[6] Cytoplasmic membrane dynamics may be the causes of chromatin removal from nucleus. Utani *et al*. described a phenomenon that explained the cytoplasmic blebbing and the chromatin pulling out of the nucleus.[12] They stressed that the phenotypical assessment of the cancer cell phenotype was mostly dependent on this mechanism.[12] The suggestion was in concordance with our findings. It has been shown that p53 inactivation results in nuclear budding in S-phase.[14] Abnormal nuclear structures such as micronuclei and nuclear

blebs were also observed at anaplastic giant cell carcinoma of thyroid.[15] In these tumors, p53 and Ki67 overexpression were also seen.[15] Krasovec *et al*. reported that nuclear budding was a rare finding among cytological findings of thyroid medullary carcinoma and in other thyroid tumors, too.[15]

In this study, granular chromatin was seen both in Hürthle cell neoplasia and oncocytic papillary carcinomas. Renshaw reported granular nature of chromatin in Hürthle cell neoplasia more often than in oncocytic papillary carcinoma, but not found statistically significant.[16] The important parameters in univariate analysis such as patient age and ultrasonographic hypoechogenicity signify the importance of clinical findings in Hürthle cell lesions. Lee *et al*. found that ultrasonographic hypoechogenicity was a significant finding in predicting malignant outcome in a study with 143 cases suspicious for Hürthle cell neoplasia cytology.[17]

In our study, increased Hürthle cell component in smears was found to be important in neoplastic and malignant outcome by univariate analysis despite other reports that found only in neoplastic outcome.[19,20] Parameters that were found significant in our univariate analysis such as small cell dysplasia, large cell dysplasia, dishesion, and Hürthle cell preponderance were also found as malignant outcome criteria in the study of Wu *et al*.[1]

Papillary structure, nuclear membrane irregularity, and nuclear enlargement could not reach importance in multivariate analysis. Consistent with general knowledge, nuclear pseudoinclusions and nuclear grooves were found insignificant.[17] Increased lymphocytes and colloid were related to non-neoplastic outcome. Wu *et al*. compared 12 HCC with 8 benign non-neoplastic Hürthle cell lesions and reported that the presence of abundant colloid and lymphocytes were reliable findings for the non-neoplastic Hürthle cell lesions.[1] Because our Hürthle cell carcinoma and Hürthle cell adenoma cases were limited in number, it was not possible to comment on the differential diagnosis of these groups.

While re-evaluating FNAC slides of the 128 cases, most important problem we encountered was to determine which cells were Hürthle and which were not. Hürthle cells are polygonal, big, and relatively uniform, with granular cytoplasm and distinct margins. The nucleus is large, generally eccentric, either single or double, and sometimes pleomorphic. The nucleolus is prominent. There are two kinds of cells that can be confused by Hürthle cells: 1. follicular epithelial cells like Hürthle cells with enlarged and granular cytoplasm, 2. Hürthle-like tumor cells in PTC, which are cells with granulated cytoplasm but have more distinct cytoplasmic borders. The major problem encountered by the scientists who study the biology of Hürthle cells is the differentiation of Hürthle cells from the mitochondrion-rich oncocytic cells.[21] Hürthle cells are described as the cells with the large eosinophilia granular swollen cytoplasm because of the accumulation of the mitochondria and with prominent nucleoli and hyperchromatic nuclei. Full-blown Hürthle cells do not show polarity and tend to form trabecular, sheets, or solid clusters. Mitochondrion-rich cells have papillary pattern and do not loose their polarity.[21]

Second noticeable point in our study is the high PTC diagnosis ratio in cytopathological correlation. In the study of Pu *et al*., all 27/87 cases between SFN, Hürthle cell type cytology reported as malignant after resection.[22] These malignant cases were composed of 15/27 (56% HCC, 12/27 (44%) PTC). In the study by Kauffman *et al*., 21/110 (19%) cases reported as malignant among patients with Hürthle cells in FNA of thyroid nodules. 13/21 (76%) cases were PTC.[23] The morphologic mimicry is the reason for this discrepancy. In our previous study, half of the cases diagnosed as PTC in histology were in the AUS/FLUS category.[3] After examination of the FNA preparations of these AUS/FLUS category cases, slides were seen to be hypocellular and tumor cells had Hürthle-like granular cytoplasm. It was observed that these cells had no strikingly atypical PTC nuclear criteria. Máximo *et al*. have pointed out that it is difficult to evaluate the PTC nuclear atypia criteria in Hürthle cells.[21] The typical nuclear features of PTC were obscured by hyperchromatism.[21] Nuclear atypia criteria and cytoplasmic features should be evaluated with more care in the follicular epithelial cells having Hürthle-like cytoplasm. In our study, small cell dysplasia, presence of papilla formation, squamoid-like cytoplasm, granular chromatin, irregular, nuclear membrane ($P < 0.001$) were statistically meaningful criteria for differential diagnosis for PTC with Hürthle-like cells. These features in the setting of Hürthle cell presence may be used in favor of papillary carcinoma diagnosis.

Another reason of high incidence of PTC in the Hürthle cell group was evaluation by MGG stain. Yang *et al*. performed hemolysis, rehydrated air dried slides and stained with Papanicolaou stain. In their study, 129 Hürthle cell lesions were included. After histopathological examination, 2 of them turned out to be oncocytic follicular variant papillary carcinoma.[24]

In our study, $BRAF^{V600E}$ mutation was found in one-third of the PTC cases. Three cases which showed mutation, were classified as classical variant of PTC. Oncocytic variants did

not show *BRAF*^{V600E} mutation. In the review of Nikiforov *et al*., *BRAF*^{V600E} mutation and histological variants were linked. *BRAF* ^{V600E} mutation was seen in 60% of classical variant, 100% of tall cell variant, and 10% of follicular variant of PTC.[25] We did *BRAF* mutation analyses because we wanted to learn whether and how much helpful it may be to do this test on cytological specimens containing Hürthle-like cells to predict surgical outcome such as PTC. As *BRAF* mutation testing was positive in only a limited number of cases, it may be helpful to diagnose FNA with Hürthle-like cells as PTC cells at least in some cases.

Conclusion

In conclusion, using cytomorphological, clinical, radiological features and *BRAF* mutation status, cytopathologists are likely to predict the neoplastic outcome in the presence of Hürthle and/or Hürthle-like cells in thyroid FNAs. Nuclear budding was significant and remarkable clue either neoplastic or malignant outcome. Nuclear budding, granular chromatin, and hypoechoic nodules in ultrasonography were significant independent factors for increased malignancy risk of PTC cases containing Hürthle cell-like cells. In addition, there are helpful utilizable criteria for accurate diagnosis in PTC cases having mild cytological atypia and Hürthle-like features. It was shown that investigating *BRAF*^{V600E} mutation in cytological material may also be helpful for correct diagnosis of PTC in the presence of Hürthle-like cells.

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Conflicts of interest

There are no conflicts of interest.

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