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Original article

Value of additional von Kossa staining in thyroid nodules with echogenic spots on ultrasound

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ABSTRACT

Purpose: To evaluate the clinical value of additional von Kossa staining on cytology in thyroid nodules with echogenic spots on ultrasound (US).
Materials and methods: 342 thyroid nodules were analyzed for cytology and von Kossa staining was performed to detect microcalcifications. We compared diagnostic performances and accuracies of FNA and FNA with von Kossa staining to detect malignancy. We evaluated associations of aggressive pathologic features and von Kossa positivity in the surgically confirmed papillary thyroid carcinoma (PTC) group.
Results: Two hundred and thirty two (67.8%) nodules were malignant and 110 (32.2%) were benign on cytopathology. Compared to cytology alone, additional von Kossa staining slightly improved sensitivity from 89.7% to 90.9% ($P = 0.081$). In 207 cases of surgically confirmed PTC, von Kossa positivity was an independent predictor of central lymph node metastasis in PTCs by multivariate analysis (odds ratio, 2.218; $P = 0.021$).
Conclusions: Adding von Kossa staining to cytology improved the sensitivity of thyroid nodules with echogenic spots on US. Positive von Kossa stains in PTCs may also be useful in predicting central lymph node metastasis.

1. Introduction

As high resolution ultrasound (US) is increasingly used in medical examinations, the number of both thyroid nodules and thyroid carcinomas has grown steadily [1–3]. Known US features of malignancy are marked hypoechogenicity, microcalcifications or mixed calcifications, noncircumscribed margins, and nonparallel shape [4,5]. Among these findings, microcalcifications are a highly specific feature of malignancy. Microcalcifications appear as echogenic spots about 1 mm in diameter with or without acoustic shadows or as tiny acoustic shadows [6,7]. Both calcium phosphate and calcium oxalate are seen as "microcalcifications" on thyroid US. Calcium oxalate is a birefringent crystal and is seen mainly in normal thyroid tissue, nodular goiter, and follicular neoplasm, but is extremely rare in papillary thyroid carcinoma (PTC) [8]. On the other hand, calcium phosphate has been commonly detected in benign and malignant thyroid nodules and is further categorized as "psammoma bodies (PBs)", "psammomatoid bodies", and "dystrophic calcifications" [9]. Of these, PBs, are well known as a characteristic of PTC. They can usually be distinguished from the aforementioned other concretions (psammomatoid bodies and dystrophic calcifications) by typical concentric lamination, and are distinguished from oxalate crystals by the lack of birefringence [7–13].

PBs are found in up to 50% of PTCs [14] and they are significantly associated with aggressive PTC features and consequently, patient prognosis, such as tumor recurrence and distant metastasis [13,15–18]. The von Kossa staining is a simple and inexpensive method that can detect calcium phosphate in hyaline globules and the presence of calcium phosphate is considered to be an initial precursor of PBs [14,19,20]. Therefore, von Kossa staining can determine the presence of malignant microcalcifications associated with PBs. To our knowledge, there has been no investigation on the role of von Kossa staining when added to cytologic diagnosis for thyroid malignancies. The purpose of our study was to evaluate

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the clinical value of additional von Kossa staining to cytology in thyroid nodules with echogenic spots on US.

2. Materials and methods

This study was approved by the Institutional Review Board of Severance Hospital, Seoul, South Korea, and all participants provided written informed consent prior to study enrollment.

2.1. Study population

From July 2013 to December 2014, 4969 thyroid nodules in 4407 patients underwent US-guided fine-needle aspiration (US-FNA) at our institution, of which 381 thyroid nodules in 372 patients had echogenic spots on US. Among them, 39 nodules were excluded because they did not undergo cytopathologic confirmation. Therefore, a total of 342 nodules in 335 patients (77 men and 258 women) were included in this study. The mean age of the patients at diagnosis was 47.7 years (standard deviation (SD), 13.1 years; range, 21–86 years). The mean size of the nodules was 13.8 mm (SD, 9.0 mm; range, 3–57 mm).

2.2. Imaging evaluation

All patients underwent an US examination of the thyroid by one of 4 board-certified radiologists specializing in thyroid imaging. A 5- to 12-MHz linear array transducer was used for the examination with US units (iU22, Philips Medical system, Bothell, WA).

The nodule size was defined as the largest diameter on US. US features of each thyroid nodule were described according to internal components, echogenicity, margin, calcifications, and shape [5]. According to published criteria, microcalcifications or mixed calcifications, marked hypoechogenicity, noncircumscribed margins, and nonparallel shape were defined as suspicious US features [5]. Nodules with any of these features were considered suspicious malignancy and US-FNA was recommended.

2.3. US-guided fine-needle aspiration (US-FNA)

All US-FNAs were performed by the same radiologist who performed the US examination. US-FNA was performed with a 23-gauge needle attached to a 2-mL disposable syringe without an aspirator. Obtained samples were expelled on glass slides, smeared, and immediately placed into 95% alcohol for Papanicolaou staining and von Kossa staining. The remaining material in the syringe was rinsed in normal saline for cell block processing. Cytopathologists were not present during US-FNA and cytology slides were reviewed by an experienced pathologist. According to the Bethesda System for Reporting Thyroid cytology, cytologic reports were divided into the following six categories; non-diagnostic, benign, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), suspicious for follicular neoplasm, or suspicious for a Hurthle cell neoplasm, suspicious for malignancy, and malignancy [21].

Calcifications were divided into PBs and dystrophic calcifications by an experienced cytopathologist (K.H.J.) who was always unaware of the final pathologic results. When a nodule had dystrophic calcifications as well as PBs, we listed it as with PBs. Subsequently, the uncovered smear slides were stained by von Kossa staining. The slides were incubated with an aqueous solution of 5% silver nitrate for 60 min at room temperature under sunlight until they took on a dark brown color. Following a DW rinse, the slides were incubated with a 5% sodium thiosulfate solution for 5 min. Calcium salts were often stained black or brown-black.

2.4. Surgery

Out of the total 342 nodules, 222 nodules underwent surgery. Of these, 216 nodules were malignancies. Prophylactic central node dissection was performed in all cases, and modified radical neck dissection was selectively performed in cases suspected or cytologically confirmed as lateral lymph node metastasis. For final pathologic reports, medical records of each case were reviewed.

2.5. Data and statistical analysis

We evaluated the clinical value of the von Kossa stain as a preoperative diagnostic factor for thyroid cancer using the cytopathological diagnosis as the “gold standard”. Of 381 nodules, we included cytopathologically confirmed thyroid nodules with the reference criteria for the “gold standard” according to the following conditions; (1) 222 nodules underwent surgery, (2) 114 nodules confirmed as “benign”, or “malignant” on the first FNA, (3) 3 nodules confirmed as “AUS/FLUS” on the first FNA and confirmed as “benign” on follow-up FNA, and (4) 3 nodules confirmed as “nondiagnostic” on the first FNA and confirmed as “benign” on follow-up FNA.

Clinicopathologic characteristics and the von Kossa staining status were compared with the *t* test for continuous variables, and the χ^2 or Fisher’s exact test for categorical variables on a subject-level analysis and the Generalized Estimating Equations (GEE) method for clustered data on a nodule-level analysis.

Malignancy and suspicious for malignancy on cytology results were classified as the “positive” cytology group. Nondiagnostic, benign and AUS/FLUS on cytology results were classified as the “negative” cytology group. To calculate the diagnostic performances of FNA, we compared the cytologic results to the “gold standard”. We defined the true-positives (TP), true-negatives (TN), false-negatives (FN) and false-positives (FP) as follows; TP, “positive” cytology and “positive” gold standard result; TN, “negative” cytology and “negative” gold standard result; FN, “negative” cytology but “positive” gold standard result; FP, positive cytology but “negative” gold standard result. To calculate the diagnostic performances of FNA with von Kossa staining, a nodule was classified into the “positive” group when either FNA or von Kossa staining showed positive results and we compared the diagnostic performances of FNA alone and FNA with von Kossa staining to detect malignancy, using the GEE methods. The accuracies of FNA alone and FNA with von Kossa staining were assessed with the area under the ROC curve (AUC). The AUC was calculated with the bootstrap percentile method with 1000 bootstrap replications.

Multivariate logistic regression analysis was used to evaluate independent associations of aggressive pathologic features (multifocality, extrathyroidal extension, lymph node metastasis, and advanced stage) with adjustment for age, gender, tumor size, multifocality, extrathyroidal extension, lymph node metastasis, and von Kossa positivity. Odds ratio and 95% confidence intervals were calculated to determine the relevance of all potential predictors of outcome. *P* values below 0.05 were considered statistically significant. Statistical analysis was performed by a software program (SAS, version 9.2, SAS Inc., Cary, NC, USA).

3. Results

Of 342 nodules, 232 (67.8%) nodules were malignant and 110 (32.2%) were benign on cytopathology (Table 1). A total of 222 nodules underwent surgery, and 216 nodules were malignant (Fig. 1). Patients with cytopathologic malignancy were significantly younger than patients with benign cytopathology results ($P < 0.001$), but gender was not significantly different