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Original contribution

BRAF^{V600E} mutation analysis of liquid-based preparation–processed fine needle aspiration sample improves the diagnostic rate of papillary thyroid carcinoma☆

Hyeyoon Chang MD[a], Hyunjoo Lee MD, PhD[b], Sun Och Yoon MD, PhD[c], Hankyeom Kim MD, PhD[a], Aeree Kim MD, PhD[a], Baek-hui Kim MD, PhD[a,*]

[a]*Department of Pathology, Korea University Medical Center, Guro Hospital, Seoul, Korea 152-703*
[b]*Department of Pathology, Kangwon National University Hospital, Republic of Korea 200-722*
[c]*Department of Pathology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea 135-720*

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Summary Early detection and diagnosis of papillary thyroid carcinoma are important for successful management of patients. Liquid-based preparations (Thinprep) of fine needle aspirations from thyroid nodules are now widely used and are replacing conventional smears because residual samples can be used for ancillary tests. Detection of the *BRAF*^{V600E} mutation in cytology specimens could aid in the diagnosis of papillary thyroid carcinoma. We, therefore, analyzed the cytologic features and *BRAF*^{V600E} mutation status of thyroid liquid-based preparation–fine needle aspiration samples. A total of 191 histologically confirmed thyroid liquid-based preparation–fine needle aspiration specimens were selected. We analyzed cytomorphological features and *BRAF*^{V600E} mutation status in both liquid-based preparation–fine needle aspiration samples and the corresponding formalin-fixed, paraffin-embedded tissues. The Seeplex BRAF ACE detection kit (Seoul, Korea), melting curve analysis with SYBR green, and sequencing analysis were used to detect *BRAF*^{V600E} mutations. Of 191 patients, 126 had histologically confirmed papillary thyroid carcinoma, whereas the remaining 65 lesions were benign lesions and carcinomas of other types. The sensitivity of liquid-based preparation alone for diagnosis of papillary thyroid carcinoma was 71.4%. When *BRAF*^{V600E} mutation analyses results were considered in conjunction with the cytologic diagnosis, the diagnostic sensitivity for detecting papillary thyroid carcinoma increased to 84.9% regardless of the method used to detect *BRAF* mutations. *BRAF*^{V600E} mutation analysis using residual liquid-based preparation cytologic samples is, therefore, a powerful additional diagnostic tool for diagnosis of papillary thyroid carcinoma.

1. Introduction

The prevalence of thyroid nodules is between 4% and 7% in the general population, and the detection rate of thyroid nodules has increased dramatically in recent years because of advances in ultrasonographic techniques for detecting small

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* Corresponding author.
E-mail address: maelstrom@naver.com (B. Kim).

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nodules during general checkup [1]. Papillary carcinoma is the most common thyroid malignancy, accounting for 80% of all thyroid neoplasms [2]. Given these trends, correct diagnosis of papillary thyroid carcinomas (PTCs) is essential.

Fine needle aspiration (FNA) using ultrasonography is the most useful tool currently available for the diagnosis of thyroid nodules [3,4]. However, cytomorphological examination of FNA alone does not discriminate between nonneoplastic and neoplastic lesions in a large number of samples [5].

Two methods are currently used to prepare thyroid FNA samples: the conventional smear (CS) method and a novel liquid-based preparation (LBP) method (Thinprep) [6]. LBP differs from CS in that residual material and cell block slides can be made, facilitating various ancillary tests such as molecular and immunohistochemical studies without sample destruction so that cytologic diagnoses can still be made.

Mutations in *Ras* [7], *RET*/*PTC* rearrangements [8], and *PAX8* (paired box gene 8)-peroxisome proliferator-activated receptor γ fusions [9] are known to be related to the development of thyroid carcinoma. However, the $BRAF^{V600E}$ mutation is frequently and specifically found in papillary carcinomas; this mutation has an average prevalence of 44% in sporadic PTCs but is rarely observed in other types of thyroid tumors [10]. Although controversial, some recent studies have demonstrated a close association between the $BRAF^{V600E}$ mutation and poorer prognostic factors of thyroid carcinoma such as extrathyroid extension, lymph node metastasis, and advanced TNM stage [11,12]. We hypothesized that $BRAF^{V600E}$ mutation analysis of LBP-processed FNA samples would improve the diagnostic rate of PTCs and, therefore, help determine treatment strategies and predict prognosis. To test our hypothesis, we analyzed cytologic features and the $BRAF^{V600E}$ mutation status of thyroid LBP-FNA samples and matching formalin-fixed, paraffin-embedded tissue counterparts using 3 different methods: a commercial *BRAF* polymerase chain reaction (PCR) kit, melting curve analysis (MCA) using SYBR green, and sequencing analysis. We also examined the association between the presence of the $BRAF^{V600E}$ mutation and poor prognosis.

2. Materials and methods

2.1. Patients

Patients who underwent thyroid FNA using the LBP method at the Korea University Guro Hospital from September 2008 to August 2009 and who had surgery (total thyroidectomy, lobectomy with or without lymph node dissection) after FNA were included in this study regardless of tumor histologic type. Among these, we excluded patients without formal, documented, informed consent. We obtained thyroid LBP-FNA specimens and their correlated surgical specimens from the Korea Lung Tissue Bank (Seoul, Korea). Ultimately, the thyroid nodules of 191 patients (191 thyroid nodules) were subjected to $BRAF^{V600E}$ mutation analysis. We also analyzed the characteristics of tumors, namely, multiplicity, bilateral lobe presentation, size, lymph node invasion, minimal extrathyroid extension, metastasis, recurrence rate, surgical margin involvement, and sex of the patient. This study was approved by the Institutional Review Board of Korea University Guro Hospital, Seoul, Korea (institutional review board approval no. KUGGR-2009-030).

2.2. DNA extraction: cytology

After preparing slides and cytologic examination, residual materials in preservative fluid (ThinPrep PreservCyt solution; Hologic Corp., Marlborough, MA, USA) were stored at room temperature for periods ranging from several months to 1 year at the most. This material was centrifuged at 4000*g* for 5 minutes, after which the supernatant was carefully removed, and the remaining material was used for DNA extraction. DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Chatsworth, CA) according to the manufacturer's protocol. After DNA extraction, we measured the concentration of the DNA samples using a spectrophotometer and diluted all samples to a working concentration of 10 μg/mL.

2.3. DNA extraction: surgical specimens

Five to 10 sections that were 10-μm thick were cut from the formalin-fixed, paraffin-embedded tissue blocks. The sections were dewaxed and stained with hematoxylin and eosin. Sections were covered with coverslips, and the areas of representative nodules were marked with a pen under a microscope. Representative sections from tumors or benign nodules were dissected on the glass using a clean blade and placed in a 1.5-mL tube. DNA was extracted with the QIAamp DNA Mini kit according to the manufacturer's instructions. After DNA extraction, the concentration of the DNA samples was measured using a spectrophotometer.

2.4. Cytopathologic examination

All cytologic and histologic slides were reviewed. The cytologic slides were reviewed for background, the morphology of cell clusters, nuclear features, and cytoplasmic features. Five categories were used as diagnostic criteria [13]. The minimum criterion to be regarded as a satisfactory sample was inclusion of at least 6 clusters with 10 follicular epithelial cells in each cluster. A diagnosis of "negative for malignancy" was made when benign diseases such as nodular hyperplasia and thyroiditis were suspected. The "atypical cellular lesion" category included cases with uncertain malignancy. "Suspicious for malignancy" and "malignant cells present" categories included cases with malignant features. The histologic slides were reviewed by 2 cytopathologists; and in cases of uncertain diagnoses, immunohistochemical stains for galectin-3, a high–