ULTRASOUND-GUIDED FNA COMBINED WITH BRAFV600E IN DIAGNOSING THYROID NODULES

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ABSTRACT

Objective: This study aims to study the clinical value of fine needle aspiration (FNA) combined with BRAFV600E gene mutation detection in diagnosing thyroid nodules.

Methods: A total of 153 patients with thyroid nodules were performed cytological test by FNA and detected the gene mutation of puncture samples by RAFV600E, as well as compared with the postoperative pathological results.

Results: Among 153 malignant cases, 132 cases were positive and 21 cases were negative for FNA, and 99 cases had BRAFV600E mutation. Among 22 benign cases, 4 cases were positive and 18 cases were negative for FNA, and no case had BRAF V600E mutation. Compared with the postoperative pathological results, the sensitivity was 86.0%, the specificity was 82.3%, and the accuracy was 87.4% for FNA. The sensitivity of BRAF mutation detection was 64.8%, the specificity was 100.0%, and the accuracy was 64.8%. The sensitivity, specificity, and accuracy of FNA-BRAFV600E in diagnosing thyroid cancer were 90.2% (141/153), 82.3% (18/22), and 85.4% (149/175), respectively, which were higher than those obtained by single BRAFV600E gene mutation detection, and the differences were statistically significant (P<0.05). The sensitivity of FNA-BRAFV600E mutation detection was higher than single FNA (P<0.05).

Conclusions: The combination of FNA-BRAFV600E mutation detection can improve the diagnostic sensitivity and specificity against benign and malignant thyroid nodules.

Keywords: Thyroid nodules, fine needle aspiration cytology, BRAF gene mutation detection.

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Introduction

In recent years, the detection rate of patients with thyroid nodules increases year by year, and thyroid nodules have become frequently-occurring and common disease in endocrine diseases(1). The detection rate of thyroid nodules in normal adults is as high as 40%, and about 19%-67% in the elderly and in females, among which the detection rate of malignant nodules is about 5%-15%(2). About 5%-7% of patients with thyroid nodules are accompanied by the corresponding symptoms(3,4). Therefore, early diagnosis of thyroid nodules and discovery of its causal pathogen have become crucial.

With the development of medical technologies, high-resolution ultrasound has become an important detection method against thyroid nodules(5). However, the clinical evaluation of thyroid nodules depends on fine needle aspiration (FNA). As a reliable and nearly noninvasive examination method, FNA is of great importance in the qualitative diagnosis of thyroid nodules. According to benign and malignant grading, FNA is divided into 6 grades(6). The "Guidelines for diagnosis and treatment of thyroid nodules and differentiated thyroid cancer" has pointed out that imaging-suspected malignant nodules should be performed FNA. However, there are still 15%-30% of the FNA results existing uncertainty(7).

Therefore, FNA tests still have false negative results(8), and molecular diagnostic techniques in recent years have become an important comple-
ment to imaging and FNA, which effectively raise the detection rate of thyroid cancer. It has been reported that\(^9,10\) BRAF\(^{V600E}\) gene mutation is an important molecular marker to promote the formation and progression of thyroid cancer, with the detection rate of thyroid cancer as about 29%–83%. Furthermore, BRAF\(^{V600E}\) is also a molecular marker for evaluating the prognosis of thyroid cancer\(^11\). So, this study investigated and analyzed the results of FNA-BRAF toward the patients with thyroid nodules, aiming to explore the application values of FNA-BRAF gene mutation in diagnosing benign and malignant thyroid nodules and to compare the results with single examination method.

Materials and methods

Subjects

The patients admitted into the department of thyroid surgery, undergone ultrasound-guided FNA-BRAF\(^{V600E}\) gene mutation detection, and confirmed by surgical pathology from August 2014 to August 2017 were selected, including 49 males and 126 females (a total of 175 nodules), aging from 27 to 72 years, with the mean age as \((42.6\pm8.5)\) years and the nodule size as 1.1–2.4cm. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Jilin University. Written informed consent was obtained from all participants.

Procedures of cytological puncture

The Mindray DC-7Exp ultrasonic diagnostic apparatus was used (Mindray, Shenzhen, China), with linear probes and the frequency as 5–12MHz. Each patient was placed in the supine position, with the shoulder uplifted and the head-neck in the hyperextension position, for routine color Doppler ultrasound examination of the thyroid tissue to observe the nodular morphology, boundary, internal echo, and internal and peripheral blood flow; the nodules and sites for the puncture were then determined, which were usually chosen from the site with the lowest nodular echo or calcification so as to increase the possibility of obtaining positive samples. After determined the puncture path, routinely disinfected the skin of puncture point, and paved the sterile towel, one sterile probe cover-wrapped TSK disposable 21G manual suction biopsy needle (TSK, Tochigi-Ken, Japan) was inserted, under the color Doppler ultrasound guidance, into the site locally anesthetized by lidocaine. After withdrew the piston rod of the cell needle, the suction was performed under negative pressure at different sites (more than 5 times, during which process the needle tip should be ensured to be always located inside the nodule). After sampling, the negative pressure was slowly removed, and the aspirate from the puncture needle was evenly sprayed on glass slides, followed by 95%-ethanol fixation and cytological examination. After the puncture, the puncture site was locally pressure-bandaged using sterile gauze and pressed for 20 min.

Diagnostic methods of cytology

All the cytological results were interpreted by the same senior pathologist.

BRAFV600E gene mutation detection

The nodules were re-punctured using the same method as cytology puncture to get the specimens, which were stored in EP tubes at -4°C. The BRAF\(^{V600E}\) gene mutation was detected by polymerase chain reaction (PCR) fluorescence probes using the blood/cell/tissue genomic DNA extraction kit (Tiangen Biotechnology Co., Ltd., Beijing) according to the following steps:

- The cells obtained by puncture were added into the buffer GA to make a suspension;
- The suspension was added the proteinase K and mixed well;
- The buffer GB was then added, mixed well, and stood at 70°C for 10 min;
- After stirred thoroughly with anhydrous ethanol for 15 min, the suspension was briefly centrifuged to remove the water droplets on the interior tube wall;
- The resulted solution was then added into adsorption column CB3, centrifuged at 12000 rpm for 30 sec, and drained the waste;
- After added Buffer GD into CB3, the mixture was centrifuged at 12000 rpm for 30 sec and discard the waste;
- The rinsing liquid PW was then added, followed by 30-sec centrifugation at 12000 rpm, the waste liquid was discard;
- The mixture was then centrifuged at 12000 rpm for 2 min, discarded the waste, and allowed to stand at room temperature for a few minutes;
- Certain amount of elution buffer TE was added into adsorption column CB3, stood for 2-5 min at room temperature, and centrifuged for 2 min at 12000 rpm, and the solution was then collected.
The BRAF\textsuperscript{V600E} mutation detection kit (Wuhan Youzhiyou Medical Technology Co., Ltd.) was used to prepare the reaction solution targeting the specific primer of V600E mutation on the 1799 nucleotide of BRAF gene, as well as the probes and PCR buffer. The intensity of fluorescence signal was tested using one fluorescence quantitative PCR instrument, and the test results were then qualitatively analyzed according to the fluorescence thresholds delineated based on the amplification curve.

**Statistical analysis**

SPSS19.0 statistical software was used for the analysis; the comparison of the rate used the $\chi^2$ test, with $P<0.05$ considered as statistical significance.

**Results**

**Surgical pathologic results**

A total of 175 thyroid nodules were all surgically removed from the patients, and the postoperative pathological results were as follows: the 153 malignant cases included 146 cases of papillary carcinoma and 7 cases of follicular carcinoma; the 22 benign cases included 2 cases of Hashimoto’s thyroiditis, 2 cases of adenoma, and 18 cases of nodular goiter (Table 1).

**Comparison of FNA with postoperative pathology**

The sensitivity, specificity, and accuracy of preoperative FNA in diagnosing thyroid cancer were 86.0% (132/153), 82.3% (18/22), and 87.4% (66/87), respectively (Table 2).

**Comparison of BRAF\textsuperscript{V600E} mutation detection with postoperative pathology**

Among the 153 patients with pathologically diagnosed malignancy, 99 cases were detected BRAF\textsuperscript{V600E} mutation preoperatively, and the former 99 patients were confirmed as papillary carcinoma.

**Comparison of FNA-BRAF\textsuperscript{V600E} mutation detection with postoperative pathology**

The sensitivity, specificity, and accuracy of FNA-BRAF\textsuperscript{V600E} mutation detection in diagnosing thyroid cancer were 90.2% (141/153), 82.3% (18/22), and 85.4% (149/175), respectively, higher than single BRAF\textsuperscript{V600E} mutation detection or single FNA, and the differences were statistically significant ($P<0.05$) (Table 4).

**Discussion**

The detection and qualitative diagnosis of thyroid nodules mainly rely on imaging results, of which ultrasound has the most value and diagnostic significance; ultrasound-guided FNA can obtain preoperative pathological results, so it has become the most commonly used method for clinical iden-
tification of benign and malignant thyroid nodules (Figure 1)(12). Miller(13-16) investigated the pathological specimens of 5000 patients with thyrocytes and put forward cytopathological diagnostic criteria of a variety of thyroid diseases. In 2007, the thyroid FNA conference held by the National Cancer Institute (NCI) developed unified standards of thyroid cytology and diagnostic terms to make the clinical application of thyroid FNA more extensive(17,18).

FNA refers to using fine needle to aspirate micro-amount cells from the thyroid tissue for preparing smears so as to preliminarily screen benign and malignant nodules, and its identification accuracy and cost-effective against benign and malignant nodules are high(19). As for experienced physicians, the sensitivity, specificity, positive predictive value, false negative rate, and false positive rate of preoperative FNA in assessing thyroid nodules can be 83% (fluctuating between 65% and 98%), 92% (fluctuating between 72% and 100%), 75% (fluctuating between 50% and 96%), 5% (fluctuating between 1% and 11%), and 5% (fluctuating between 0% and 7%), respectively(20).

However, there are still some limitations: If the pathology is not typical, without papillary structure change or only slight changes of nucleus, it may result in false negative results of papillary carcinoma; due to the limited amount of specimens obtained by fine needle aspiration, as well as the tissue morphology and interstitial structure of the specimens are mostly or completely destructed, it can’t reflect the invasion extent of extravascular tumor or capsule, can’t identify follicular adenoma from follicular carcinoma; if the intra-nodular blood flow is rich, too many red blood cells may contaminate the smear; in addition, the experience of the operating physicians and pathologists also has a large impact on the FNA results. This study showed that the sensitivity of FNA alone was 75.0%, and 10 cases of papillary carcinoma were diagnosed as benign due to not obtaining the malignant nodular lesions during the process of puncture; 5 patients with false positive cytopathology were found atypia or papillary proliferation and diagnosed as (or tend to) papillary carcinoma; in order to confirm the diagnosis, some patients were recommended thyroidectomy, but the postoperative pathology did not meet the diagnostic criteria(21). Therefore, the diagnostic rate of thyroid nodules by FNA is not completely satisfactory yet.

The BRAFV600E mutation is the most common genetic mutation against thyroid cancer (Figure 2). American scholar Patel(22) has proven that even in stage I papillary carcinoma, BRAF gene mutation is also significantly related to its recurrence. Related literatures have reported that the detection rate of BRAFV600E mutation in papillary carcinoma is 29% to 83%, and about 24% in undifferentiated carcinoma(23-25). Its expression in other pathological types of thyroid cancer and benign thyroid lesions has not been reported yet.

The mutation of BRAFV600E is closely related to the occurrence of papillary carcinoma. It has been reported that 29%-83% of papillary carcinomas can detect the BRAFV600E mutation, but it’s not expressed in benign lesions. In this study, the 99 patients with BRAFV600E mutation revealed preoperatively were confirmed as papillary carcinoma by postoperative pathology, but no BRAFV600E mutation was found in the 22 patients with benign lesions, indicating that BRAFV600E mutation is a genetic event unrelated to benign lesions. Although the specificity of BRAFV600E mutation in diagnosing papillary carcinoma is 100%, its low mutation rate leads to lower sensitivity and accuracy than FNA; therefore, it can’t meet the requirements of clinical diagnosis of thyroid nodules.

Because detecting BRAFV600E mutation can be
used to diagnose papillary carcinoma, the combination of FNA-based BRAFV600E mutation detection will not increase the false positive rate, and the specificities of these two have no difference. Papillary carcinoma accounts for the vast majority of clinical diagnosis of thyroid cancer (80% to 90%), so increasing the detection rate of papillary carcinoma can indirectly improve the preoperative detection rate of thyroid cancer. For the nodules with atypical pathological changes or can’t be diagnosed by FNA, the BRAFV600E mutation can be detected, and most FNA tissues with positive BRAF mutation belong to suspicious Bethesda Class V malignancy\(^{(26)}\). Therefore, compared with FNA alone, FNA-BRAFV600E mutation detection can improve the detection rate of thyroid cancer. In this study, the patients with positive results of FNA-BRAFV600E mutation were also pathologically confirmed, with the accuracy as 100%. Although detecting the BRAFV600E mutation is not meaningful for the diagnosis of benign nodules, but it can theoretically improve the detection rate of malignant nodules, thus improving the overall coincidence rate of diagnosis. The joint method in this study exhibited significantly increased examination accuracy than FNA alone, but no statistical difference can be found, and it may be related to the limited sample size, which needs further studies.

In addition, the BRAFV600E mutation is associated with the high invasiveness of papillary carcinoma toward surrounding tissues, and is a predictor of central lymph node metastasis; meanwhile, it may induce a decrease in uptake of radioactive iodine by tumor cells, thus resulting in the failure of radioiodine treatment, so it’s an impact factor that can be used to judge the prognosis. Therefore, preoperative FNA examination can not only improve the detection rate of thyroid cancer but also prompt the severity of the disease, and provide basis for prognosis assessment.

Conclusions

This study tested FNA combined with BRAF gene mutation in the patients with thyroid nodules, aiming to investigate the application value of combination of these two detection methods in determining benign and malignant thyroid nodules and to compare the results with those obtained by single detection method. The results showed that the combination of these two methods exhibited a significant improvement trend of accuracy rate than single FNA, which consistent with our previous hypothesis. However, due to the limited sample number, there exhibited no statistical difference between the combined method and single FNA, which needs further studies toward the interaction between the BRAFV600E mutation test and FNA. Detecting the BRAFV600E mutation can improve the sensitivity and accuracy of FNA examination for thyroid nodules, but the specificity did not change, and the results of joint detection were more reliable. As for the patients with unclear initial FNA results, repeated puncture may suggest a combination of methods, help to screen the cases with high-risk thyroid cancer, and guide the surgical approach and postoperative treatment.

Therefore, the combination of FNA and BRAFV600E gene mutation detection can improve the sensitivity and specificity of diagnosing benign and malignant thyroid nodules, can increase the diagnostic performance while reduce the uncertainty of the results, and can reduce the incidence of preventable diagnostic surgery.

References

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