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ORIGINAL ARTICLE

Usefulness of thyroglobulin measurement in needle washouts of fine-needle aspiration biopsy for the diagnosis of cervical lymph node metastases from papillary thyroid cancer before thyroidectomy

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Abstract In evaluating cervical lymph node (LN) metastasis from papillary thyroid cancer (PTC), ultrasonography (US)-guided fine-needle aspiration biopsy (FNAB) is very important tool. There were limited number of studies about the diagnostic value of thyroglubion measurement in FNAB (FNAB-Tg) in non-thyroidectomized patients. Therefore, in this study, the authors evaluated the role of FNAB-Tg in diagnosing cervical LN metastases in patients with PTC before thyroidectomy. A total 91 suspicious LNs of 68 patients were undergone US-guided FNAB-Tg and cytology. Any FNAB-Tg concentration above 50 ng/ml considered as positive, irrespective of thyroid gland presence. Based on the final pathology, 49 LNs were positive, and the remaining 42 LNs were negative for metastasis. The sensitivity, specificity, and accuracy of FNAB-Tg in thyroidectomized patients were 80.0, 100.0, and 88.9%, respectively. The diagnostic performance of FNAB-Tg was not compromised by the presence of thyroid gland (sensitivity, specificity and accuracy = 95.0, 90.9 and 93.2%, respectively). FNAB-Tg is useful and simple method for the diagnosis of metastatic cervical LNs from PTC. The diagnostic performance of FNAB-Tg was not compromised by the presence of thyroid gland. Therefore, FNAB-Tg could be performed actively for the LN staging of PTC.

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Introduction

The prognosis of patients who receive appropriate treatment for papillary thyroid carcinoma (PTC) is usually favourable. However, about 5–20% of patients who undergo surgery for PTC develop local or regional recurrences [1]. Loco-regional metastasis commonly involves lymph nodes (LN) in the neck [1]. Therefore, accurate diagnosis of LN involvement is an important issue for patients with PTC and is considered when deciding whether to perform neck dissection.

Because metastatic disease usually occurs in the neck, cervical US has been advocated as an important tool in the staging of PTC patients. Despite the presence of specific characteristics of malignancy, the diagnosis of cervical LN metastases of PTC can be often complex, because inflammatory lymphadenopathies are extremely frequent in cervical region [2, 3]. Therefore, US alone is not enough to distinguish a metastatic nodule from reaction hyperplasia [3]. Fine-needle aspiration biopsy (FNAB) in conjunction with US guidance can effectively diagnose PTC because its diagnosis is based upon the demonstration of classic nuclear features. Torlantano and co-workers [4] had reported that adding FNAB to US increased the accuracy in detecting metastases of PTC up to 100%.

However, in some instances the metastatic deposits of PTC in the LN may undergo degeneration and cystic change [5, 6]. In such instances the FNAB of LN even with US guidance may only show colloid type material, cellular debris and macrophages without any identifiable tumour

cells. These specimens are usually classified as 'non-diagnostic or unsatisfactory for evaluation' [6–8].

Since 1992, Pacini et al. [9] first reported the use of Tg measurements in FNAB specimens (FNAB-Tg), several studies have reported that FNAB-Tg identifies PTC metastases of the neck with higher sensitivity and specificity than FNAB-cytology [10–16]. However, there have been some concerns that the diagnostic accuracy could be compromised in the presence of thyroid gland, because that Tg may be detected in FNAB washout fluid from reactive nonmetastatic cervical LNs [9, 10]. With all these concerns, there were limited number of studies about the diagnostic value of FNAB-Tg in non-thyroidectomized patients [15, 16] and the results were controversial. Therefore, in this study, the authors evaluated the role of FNAB-Tg in diagnosing cervical LN metastases in patients with PTC before thyroidectomy.

Materials and methods

Patients

Ninety-nine consecutive patients (78 females and 21 males; mean ages ± SD, 53.4 ± 14.9 years) with suspicious cervical LNs were evaluated at the Department of Radiology, Wonkwang University Hospital, between August 2008 and December 2011. Of the 99 patients, 31 patients were excluded from the study because suspicious cervical LNs were concluded that reactive lymphadenopathy due to benign thyroid disease ($n = 28$) or metastatic lymphadenopathy of other malignancy ($n = 3$; cholangiocarcinoma, neurogenic tumour and hepatocellular carcinoma).

The remaining 68 patients were subdivided into two groups. Group A of 55 patients (42 females and 13 males; mean ages ± SD, 53.1 ± 15.3 years) had underwent US-guided FNAB for the LN staging of PTC before thyroidectomy. According to the clinical stage, these patients underwent total thyroidectomy with or without neck dissection. The interval between FNAB and thyroidectomy was 26.5 ± 31.2 days. A total number of 73 LNs were examined.

A second group B of 13 patients (10 females and 3 males; mean ages ± SD, 41.2 ± 14.2 years) had previous diagnosis of PTC and had been treated with total thyroidectomy, followed by I-131 ablation therapy (administered activity raging from 1.11 to 5.55 GBq). The interval between thyroidectomy and FNAB was 50.4 ± 29.7 months. A total number of 18 LNs were examined.

Ultrasonography and fine-needle aspiration biopsy-cytology

US examinations were performed by experienced radiologists using a 10–12 MHz linear transducer (Acuvix, Medison, Korea; iU22, Philips Medical Systems, Bothell, WA, USA). US examination was performed on both thyroid lobes and the neck, including all neck levels (level I to level VI) and the supraclavicular fossa. The US features of suspected metastatic nodes were recorded. US-FNAB was performed on metastatic nodes determined by US criteria using a free-hand technique with a 21–23 gauge needle. Biopsy needle content was expelled onto a slide and smeared using a second slide. Slides were fixed in 95% ethanol, Papanicolaou-stained, and read by an experienced cytopathologist. FNAB-cytology results were categorized as negative (non-diagnostic, reactive lymph node or some other benign lymphadenitis), or positive for metastatic papillary carcinoma.

Thyroglobulin measurement

Immediately after FNAB, the needle used was washed with 0.5 ml of normal saline solution. These washout samples were sent for FNAB-Tg assays. Serous fluid aspirates were sent directly for assaying. Tg was assayed with a monoclonal antibody immunoradiometric assay (IRMA; CIS Bio International, Gif-sur-Yvette, France). Analytical sensitivity, defined as the smallest detectable concentration different from zero with a probability of 95%, is 0.2 ng/ml. Functional sensitivity, calculated with the imprecision profule for a coefficient of variation equal to 20%, was 0.7 ng/ml. The authors considered that any Tg concentration above the reference value of serum Tg (0–50 ng/ml) as positive, irrespective of thyroid gland presence.

Statistical analysis

The sensitivity, specificity and accuracy were calculated using crosstab tables. The diagnostic performances of FNAB-cytology and FNAB-Tg were compared using chi-square test and receiver-operating characteristic (ROC) curves. Where applicable, data are presented as mean ± SD. Data were analyzed using descriptive statistics, and changes in continuous variables were analyzed using the independent-samples t test. Tests with P value <0.05 were considered significant.

Ethical statement

The ethics committee of our institute approved the study, and informed consent was obtained from all patients.

Results

A total 91 LNs of 68 patients were reviewed. Based on the final pathology, 49 LNs were positive, and the remaining 42 LNs were negative for metastasis.

Non-thyroidectomized patients (group A)

In group A, metastases were diagnosed in 41 of 73 LNs (Table 1). The sensitivity, specificity and accuracy of FNAB-Tg were 95.0, 90.9 and 93.2%, respectively. Mean FNAB-Tg value (ng/ml) was 456.0 ± 212.3 in metastatic LNs and 25.3 ± 97.4 in non-metastatic LNs ($P < 0.001$). There were 2 false-positive and 3 false-negative results. Two false-positive results were occurred from two different cervical LNs (FNAB-Tg; 372.6 and 418.9 ng/dl, respectively) of one patient with high serum Tg (682.6 ng/ml). Three false-negative results (FNAB-Tg; 14.0, 0.1 and 26.9 ng/dl, respectively) were occurred in 3 different patients, and one of them had false-negative result in FNAB-cytology examination.

On FNAB-cytology, 28, 34 and 11 LNs were diagnosed as positive, negative and non-diagnostic, respectively. The sensitivity, specificity and accuracy of FNAB-cytology were 96.4, 68.9 and 79.5%, respectively. As non-diagnostic LNs were considered as negative, 1 false-positive and 14 false-negative results were occurred.

Thyroidectomized patients (group B)

In group B, metastases were diagnosed in 8 of 18 LNs. The sensitivity, specificity and accuracy of FNAB-Tg were 80.0, 100.0 and 88.9%, respectively. Mean FNAB-Tg value (ng/ml) was 467.6 ± 300.3 in metastatic LNs and 86.3 ± 196.3 in non-metastatic LNs ($P < 0.05$). There were two false-positive results and both LNs were negative on FNAB-cytology examination.

On FNAB-cytology, 7, 10 and 1 LNs were diagnosed as positive, negative and non-diagnostic, respectively. The sensitivity, specificity and accuracy of FNAB-cytology were 100.0, 90.0 and 94.4%, respectively. As non-diagnostic LNs were considered as negative, 1 false-negative result was occurred.

Effect of the presence of thyroid gland on FNAB-Tg and FNAB-cytology

Comparing area under the ROC curves, the diagnostic performance of FNAB-Tg was not compromised in the presence of thyroid gland (Table 2). In spite of reduced specificity, the accuracy was higher in group A (93.2%) than group B (88.9%). Contrary to FNAB-Tg, the diagnostic performance of FNAB-cytology in group A was inferior that of group B. This finding was mainly caused by low specificity in group A.

Discussion

Since 1992, Pacini et al. [9] first reported the use of Tg measurements in FNAB specimens (FNAB-Tg), several studies have reported that FNAB-Tg identifies PTC metastases of the neck with higher sensitivity and specificity than FNAB-cytology [10–16]. However, there have

Table 1 FNAB-Tg and FNAB-cytology results comparing with the lymph node histopathological examination according to the thyroid gland presence

	Non-thyroidectomized patients (group A)		Thyroidectomized patients (group B)	
	Benign ($n = 32$)	Metastatic ($n = 41$)	Benign ($n = 10$)	Metastatic ($n = 8$)
FNAB-Tg[a]				
Positive	2	38	2	8
Negative	30	3	8	0
FNAB-cytology				
Positive	1	27	0	7
Negative	27	7	10	0
Non-diagnostic	4	7	0	1

[a] Thyroglobulin in washout of fine-needle aspiration biopsy

Table 2 Diagnostic performances for detecting cervical lymph node metastasis in patients with papillary thyroid cancer according to the presence of thyroid gland

	Sensitivity (%)	Specificity (%)	Accuracy (%)	Area under the ROC curve
Non-thyroidectomized patients				
FNAB-Tg[a]	95.0	90.9	93.2	0.932
FNAB-cytology	96.4	68.9	79.5	0.814
Thyroidectomized patients				
FNAB-Tg[a]	80.0	100.0	88.9	0.900
FNAB-cytology	100.0	90.0	94.4	0.938

[a] Thyroglobulin in washout of fine-needle aspiration biopsy

been some concerns that the diagnostic accuracy could be compromised in the presence of thyroid gland, because that Tg may be detected in FNAB washout fluid from reactive nonmetastatic cervical LNs [9, 10].

Despite of these concerns, there were limited number of studies about the diagnostic value of FNAB-Tg in non-thyroidectomized patients [15, 16], and the results were controversial. In the study of Kim et al. [15], they had concluded that FNAB-Tg after surgery showed higher sensitivity and accuracy than those before surgery for all five threshold values. However, in the study of Bournaud et al. [16], they had reported that low threshold of 0.93 ng/ml gives high sensitivity and specificity, even in non-thyroidectomized patients. In the present study, the diagnostic performance of FNAB-Tg was not compromised by the presence of thyroid gland. This could be accomplished by careful FNAB technique avoiding needle form the contamination of thyroid gland. Also, as the samples for FNAB-Tg had been diluted with 0.5 ml of normal saline (50- to 200-fold), the FNAB-Tg values for a negative LN would be markedly lower than that of a positive LN.

Several studies had used different thresholds of FNAB-Tg according to the presence of thyroid gland [12–15]. In the study of Boi et al. [13], they had used 36 ng/ml for patients with thyroid gland, and 1.7 ng/ml for thyroidectomized patients, as threshold of FNAB-Tg. In the study of Kim et al. [15], they had used five different thresholds of FNAB-Tg. However, in the present study, the authors had used reference value of serum Tg (50 ng/ml) as a threshold of FNAB-Tg irrespective of thyroid gland presence, according to the several previous studies [15, 17].

There were several limitations on the present study. First, the authors had not considered the presence of serum anti-Tg antibody for analysis. It is well known that serum Tg value is affected by the serum Tg antibody [1]. However, FNAB-Tg values do not seem to be substantially affected by anti-Tg antibody, and previous studies had reported this finding consistently [14–16]. Second, the number of patients recruited for group B was relatively small. However, the diagnostic performance of FNAB-Tg was comparable with other studies [10–16]. Third, the diagnostic performance of FNAB-cytology was significantly different between non-thyroidectomized and thyroidectomized patients. In the present study, considering clinical convenience, non-diagnostic results of FNAB-cytology were considered as negative. There were significantly higher non-diagnostic results in group A ($n = 11$) than group B ($n = 1$). This difference had resulted in low specificity in group A. Further studies evaluating the clinical significance of non-diagnostic FNAB-cytology results were mandatory.

In conclusion, FNAB-Tg is useful and simple method for the diagnosis of metastatic cervical LNs from PTC. The diagnostic performance of FNAB-Tg was not compromized by the presence of thyroid gland. Therefore, FNAB-Tg could be performed actively for the LN staging of PTC.

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