Original Article

Estimation of thyroglobulin in lymph node aspirates: Pilot experience from a tertiary referral cancer center

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ABSTRACT

Background: Assessment of cervical lymph node involvement in patients with thyroid cancer either during preoperative surgical mapping or detection of recurrences during follow-up is a crucial step in the management of differentiated thyroid cancers (DTCs). In most patients, fine needle aspiration cytology (FNAC) confirms the presence of metastasis in lymph node. However, in cases of paucicellular lymph node aspirate or discordant sonogram and cytology results, thyroglobulin (Tg) measurement in the lymph node aspirate (FNA-Tg) is useful and a value >1 ng/ml is considered consistent with metastatic disease. **Context:** The addition of FNAC to the US improves the specificity, but 5–10% are nondiagnostic and 6–8% rate of false-negative results. Several studies have reported that the detection of Tg in FNA-needle washes improves the evaluation of suspicious lymph nodes in patients with DTC. Data from Indian centers on FNA-Tg are limited. **Aims:** We piloted the utility of FNA-Tg in patients with sonographically suspicious cervical lymph node enlargement in the setting of suspicious thyroid nodule or in the follow-up of thyroid cancer. **Settings and Design:** Prospective data collection. **Results:** We measured Tg in 13 lymph node aspirates (12 patients, 10 females) among whom 4 patients had a total thyroidectomy and 1 had a hemithyroidectomy. Eight of the 13 lymph node aspirates had FNA-Tg values >150 ng/ml, all of them had unequivocal malignant cytology and four among them had proven metastatic DTC on surgical pathology. The median FNA-Tg of the patients with malignant cytology was 7550 ng/ml with a range of 162–30,000 ng/ml. Among the remaining 5 lymph node aspirate, 2 lymph nodes showed cytological features suggestive of reactive lymphadenitis (FNA-Tg <0.2 ng/ml) and were not operated, 1 had a high-grade malignancy consistent with anaplastic thyroid cancer (FNA-Tg <0.2 ng/ml), and 2 had nondiagnostic cytology (one had non-caseating granuloma on surgical pathology [FNA-Tg 1.3 ng/ml] and in the other patient [FNA-Tg <0.2 ng/ml] surgical intervention was deferred). **Conclusions:** FNA-Tg was concordant with positive cytology in all patients with DTC and may serve as a useful tool in patients with negative and nondiagnostic cytology to guide surgical management.

Key words: Cytology, differentiated thyroid cancer, lymph node aspirate, thyroglobulin

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Introduction

Thyroglobulin (Tg) is a 660,000 molecular weight glycoprotein produced exclusively by the follicular cells of

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the thyroid. It is secreted into the follicular lumen, where it serves as the precursor of, and storage reservoir for, thyroxine (T4) and triiodothyronine (T3). T4 and T3 are released after Tg is endocytosed and proteolytically degraded in the thyrocyte. Since Tg is produced only by follicular thyrocyte-derived cells, measurement of serum Tg levels in athyreotic patients enables detection of persistence, recurrence, or metastasis of differentiated thyroid carcinoma (DTC).[1] In addition, because of the thyroid specificity of Tg, its measurement in biopsy specimens of nonthyroidal tissues may assist in confirming and localizing metastatic disease. Papillary thyroid cancer (PTC) accounts for more than 80% of all DTC.[2] Most metastatic disease in PTC occurs in locoregional lymph nodes in the neck, which are easily examined by ultrasound. Ultrasonography (US) is highly sensitive in the detection of cervical metastases with sonographic features of abnormal metastatic lymph nodes include enlargement, loss of the fatty hilum, a rounded rather than oval shape, hypoechogenicity, cystic change, calcifications, and peripheral vascularity.[3] However, it has a low specificity (37%) because benign lymphadenopathies are extremely frequent.[4,5] Occult thyroid cancer is well-described to present as an isolated cystic mass in the lateral neck,[6,7] where thyroid sonography may be normal. In addition, cystic changes in metastatic lymph nodes can cause diagnostic problems for the cytopathologist. The addition of fine needle aspiration cytology (FNAC) to the US improves the specificity, but 5–10% are nondiagnostic and 6–8% rate of false-negative results.[5,8] Several studies have reported that the detection of Tg in FNA-needle washes improves the evaluation of suspicious lymph nodes in patients with DTC.[9-13] Data from Indian centers on FNA-Tg are limited.

Subjects and Methods

Patients

FNA-Tg was performed in 13 cervical nodes of 12 patients (10 females) with mean (+standard deviation) age of 37 + 16 years (range: 26–65 years) from July 2014 to March 2015 at the Mazumdar Shaw Medical Center. All patients were subjected to physical examination, neck US, biochemical estimation of serum thyroid stimulating hormone, and Tg. FNAC of sonographically suspicious cervical lymph nodes were sent for cytology and FNA-Tg. Four patients had previously undergone total thyroidectomy for PTC and one hemithyroidectomy. In the remaining 7 patients, FNA-Tg was performed for preoperative surgical planning. The thyroid nodule FNAC in 6 of 7 patients was highly suspicious for malignancy (Bethesda class V), while one patient had indeterminate cytology (class III). Majority of the lymph nodes (11/13) chosen for FNA were in the lateral cervical compartment while 2 patients had central level VI lymph node aspirated.

Fine needle aspiration-thyroglobulin estimation

The FNAC aspirate was sent for cytology analysis, and a dedicated aspirate was washed in 0.5 ml normal saline and sent for Tg estimation. The specimen was centrifuged and cellular debris removed, and Tg was measured in the supernatant using solid-phase chemiluminescent immunometric assay (Siemens® Immulite 1000; analytic range 0.1–1000 ng/ml and CV 7.5%). All (except one patient C) specimens were re-tested in dilution to exclude "hook effect." Cytological smears were evaluated by the pathologist, and final surgical pathology was available in patients underwent surgery.

Results

The patient details are summarized in Table 1. Eight of the 13 lymph node aspirates had FNA-Tg values >150 ng/ml, all of them had unequivocal malignant cytology and 4 among them had proven metastatic DTC on surgical pathology. The remaining patients are awaiting surgery. The median FNA-Tg of the patients with malignant cytology was 7550 ng/ml with a range of 162–30,000 ng/ml. The patient (C) with FNA-Tg of 162 ng/ml was not performed in dilution, and it is likely that hook effect might have contributed to the apparently "lower" Tg estimate. In 4 athyreotic patients, the FNA-Tg was concordant with cytology and helped plan surgical neck dissection. In the remaining 4 patients, FNAC was performed as a preoperative procedure. FNA-Tg was concordant with cytology and helped plan appropriate neck dissection along with total thyroidectomy as the initial surgery in these patients. In one of the patients (patient G), the thyroid nodule FNA Bethesda class was atypia of undetermined significance (III) and the concordant cytology, and FNA-Tg results from the lymph node changed the surgical decision from a diagnostic hemithyroidectomy to a total thyroidectomy and neck compartment dissection. Similarly, in patient (TD), preoperative imaging showed an indeterminate level III lymph node. The concordance between the negative cytology and FNA-Tg <0.2 ng/ml results helped restrict the surgery to a total thyroidectomy, and lateral neck dissection was avoided.

Among the remaining 5 lymph node aspirate, 2 lymph nodes had cytological evidence of reactive lymphadenitis, and FNA-Tg <0.2 ng/ml and were not operated. One of those patients (patient A), who had a previous total thyroidectomy and presented with iodine (I^{131})-scan negative but fluorodeoxyglucose (FDG) avid bilateral neck lymph adenopathy [Figure 1]. FNA-Tg and cytology confirmed reactive lymphadenitis from one side and metastatic lymph node on the opposite side. This converted the surgical plan from a bilateral lateral neck dissection to unilateral one.

Table 1: Details of patients who underwent fine needle aspiration cytology of lymph node and the outcomes

Name	Age (years)	Sex	Athyreotic	Thyroid nodule FNA Bethesda class	Level of cervical node aspirated	USG LN size (mm)	LN-Tg level (ng/ml)	Serum Tg level (ng/ml)	Serum anti-Tg level (IU/ml)	TSH (mIU/ml)	FNA cytology report	Surgical pathology
C	52	Female	Yes	NA***	Right supraclavicular	45 × 43	162**	-	-	-	Metastatic PTC*	Lost to follow-up
S	30	Male	Yes	NA***	Left level 4	22 × 10	10,734	-	-	8.68	Metastatic PTC*	Declined surgery
K	28	Female	No	V	Right supraclavicular	13 × 7	1.3	-	-	1.12	Nondiagnostic	Granulomas
LMR	26	Female	Yes	NA***	Left level 6	9 × 7	>30,000	302.4	35.8	0.05	Metastatic PTC*	PTC*
G	26	Female	No	III	Left level 2a	9 × 5	4363	-	-	5.23	Metastatic PTC*	PTC*
M	29	Female	No	V	Left level 4	18 × 12	18,326	-	-	2.18	Metastatic PTC*	PTC*
A	59	Female	Yes	NA***	Left level 2	10 × 6	<0.2	<0.04	1697	0.01	Reactive lymphadenitis	No surgery
A	59	Female	Yes	NA***	Right level 4	24 × 14	1936	<0.04	1697	0.01	Metastatic PTC*	PTC*
Z	50	Female	No	V	Right level 4	19 × 10	10,900	-	-	5.93	Metastatic PTC*	Lost to follow-up
ST	65	Female	No	VI	Left level 4	50 × 70	<0.2	-	-	0.53	High grade thyroid malignancy (anaplastic)	No surgery
TD	12	Female	No	V	Right level 3	25 × 15	<0.2	-	-	1.94	Reactive lymphadenitis	Not removed
V****	33	Female	No	I	Left level 6	7 × 4	<0.2	6.2	25.6	0.02	Nondiagnostic	No surgery
R	43	Male	No	V	Left level 3	55 × 31	2819	-	-	-	Metastatic PTC*	Awaiting surgery

*PTC: Papillary thyroid cancer, **Not performed in dilution, ***Not applicable since status posttotal thyroidectomy, ****Prior right hemithyroidectomy for PTC.
FNA: Fine needle aspiration, TSH: Thyroid stimulating hormone, Tg: Thyroglobulin, USG: Ultrasonography, LN: Lymph node

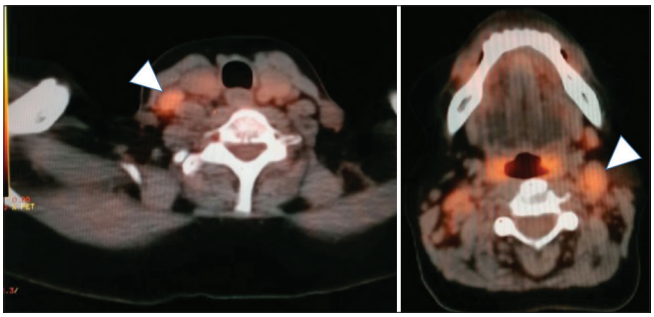

Figure 1: Fluorodeoxyglucose uptake seen on the right-sided level IV cervical lymph nodes and left-sided level II lymph nodes

Patient (ST) presented with a 4 weeks duration of rapidly enlarging thyroid swelling and FNA from thyroid nodule, and lymph node showed high-grade malignancy [Figure 2a and b]. Since the FNA-Tg < 0.2 ng/ml and the FDG avid thyroid mass with FDG avid scapular metastasis [Figure 3], and rapid clinical progression, a diagnosis of anaplastic thyroid cancer was made.

Two patient had nondiagnostic lymph node FNAC, one of them had noncaseating granuloma on surgical pathology (FNA-Tg 1.3 ng/ml), and the other patient had right hemithyroidectomy for 2.5 cm classic PTC. On

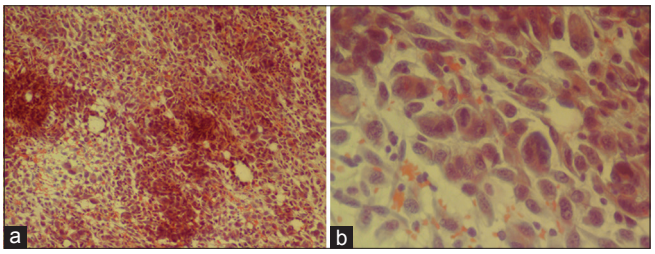

Figure 2: (a) Low power view (×10 field) - Smear shows markedly increased cellularity and is composed of sheets of moderate to highly pleomorphic cells. (b) Higher power (×20 field) - On higher power, the cells appear discohesive and show occasional large highly pleomorphic bizarre nuclei. The cytoplasm is dense and eosinophilic in appearance. No papillary nor follicular arrangement is seen. No usual nuclear features of papillary thyroid cancer such as neither nuclear grooves nor intranuclear cytoplasm inclusions are noted. No definite background colloid is seen

follow-up of the patient with hemithyroidectomy, neck US noted a 1 cm left level VI lymph node which was indeterminate on sonography, but cytology was nondiagnostic and FNA-Tg <0.2 ng/ml and hence surgery was deferred.

Discussion

The diagnosis of lymph node involvement in thyroid carcinoma is important as it changes the surgical

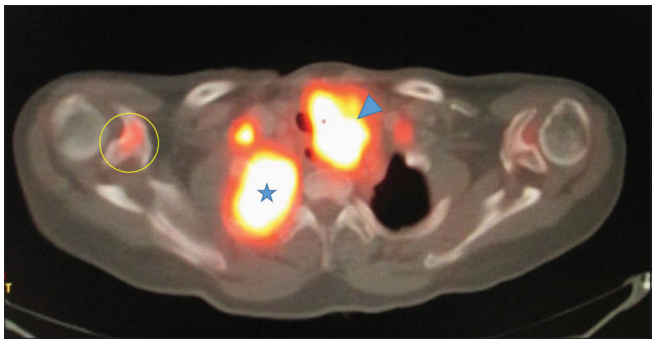

Figure 3: Fluorodeoxyglucose-positron emission tomography avid thyroid nodule (arrowhead) with lymph nodes (star) and scapular metastasis (circle)

management and prognosis of the patient with DTC. FNA-Tg is recommended as an adjunct to cytology in the diagnosis of lymph node metastasis. FNA-Tg is unaffected by the presence of serum Tg antibodies. Snozek *et al.* and Moon *et al.* reported that an FNA-Tg cutoff of 1 ng/mL provided excellent sensitivity (100% and 93%, respectively) and specificity (96% in both studies) for the detection of metastatic thyroid carcinoma in lymph nodes, respectively.[10,11] Moon *et al.* showed that combining FNA-Tg and FNA cytology showed superior diagnostic power (sensitivity, 98.4%; specificity, 94.4%) when compared with diagnostic strategy using either FNA cytology or FNA-Tg alone.[11] While the FNA-Tg value of 1.0 ng/L is the optimal cutoff for athyreotic patients, the diagnostic performance of FNA-Tg is poorer in patients before thyroidectomy.[14] FNA-Tg levels are expected to be higher in patients with thyroid glands because Tg secreted by normal thyroid can affect the FNA-Tg level due to contamination with blood during the procedure.[8,15] Studies on the FNA-Tg cutoff value in patients with thyroid glands are limited, and cutoff values have varied from 1 to 50 ng/ml.[11,12,15-17] It is also well-known that Tg synthesis is lost in dedifferentiated thyroid cancer and anaplastic thyroid cancer, and FNA-Tg is likely to be low or undetectable in these cases.[15] This is consistent with one of our patients (ST) whose clinical presentation, cytology, and imaging features was consistent with that of anaplastic carcinoma.

In our pilot study, FNA-Tg had 100% correlation with positive cytology and values are unequivocally high (>150 ng/ml) regardless of the thyroidectomy status. However, the real utility was in patients with negative cytology, where FNA-Tg <0.2 ng/ml was confirmatory of the negative cytology and reassuring in patients with nondiagnostic cytology thus helping guide surgical interventions.

Conclusions

FNA-Tg was concordant with positive cytology in all patients with DTC and may serve as a useful tool in patients with negative and nondiagnostic cytology to guide surgical management.

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Conflicts of interest

There are no conflicts of interest.

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