Dendritic Cell Expression of CD83 in Solitary Thyroid Nodules

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Abstract. **Background:** Thyroid carcinoma typically presents as a dominant solitary thyroid nodule. Fine-needle aspiration biopsy (FNAB) is considered by most to be valuable in the evaluation of palpable thyroid nodules. At present, efforts are directed to find additional markers of malignancy that may prove to be useful adjuncts to FNAB. **Aim of the Study:** To establish the role of dendritic cell (DC) expression as a complementary marker to fine needle aspiration biopsy in the diagnosis of solitary thyroid nodules. **Study Design:** As CD83 is expressed on mature dendritic cells, CD83 + DC was studied by immunohistochemistry in 43 specimens from solitary thyroid nodules removed during surgery. They were compared to CD 83 + DC in surrounding tissue from the same patients. **Results:** CD83 + DC was significantly higher in papillary carcinoma (14.287±3.76) compared to normal tissue and to follicular carcinoma. DC expression was very low in medullary carcinoma (0.023±0.03) compared to normal tissue and to other cases of benign and malignant adenoma. The sensitivity of CD83+ DC was 85% and the specificity was 82%. **Conclusion:** CD83 + DC expression in papillary carcinoma may help in its differential diagnosis of follicular carcinoma from other papillary variants. In addition to fine needle aspiration biopsy, the very low DC expression in medullary carcinoma could help in the diagnosis of such cases. Dendritic cell expression potentially represents a new marker technique to improve the sensitivity and specificity in evaluating solitary thyroid nodules.

Keywords. CD83 • Dendritic cells • Papillary carcinoma • Thyroid nodules

Introduction

Most thyroid nodules are harmless, do not cause symptoms, and do not require any treatment at all. However, approximately 5% of nodules are cancerous and therefore require attention. Cancer is less likely with multinodular goiter and more likely with single nodules.[1]

Dendritic cells (DCs) are unique antigen presenting cells (APCs) because they are the only ones that are able to induce primary immune responses, thus permitting establishment of immunological memory.[2] DCs are noted exclusively in neoplastic lesions, specifically at the periphery and within the tumor capsule. The specific distribution of DCs suggests a possible contribution to growth regulation of the thyroid neoplasm.[3]

Aim of the Work. The aim of our work is to establish the role of DC expression as a complementary marker to fine-needle aspiration biopsy (FNAB) in the diagnosis of solitary thyroid nodules. DC expression may become an ancillary tool in the diagnosis of thyroid carcinoma and in differentiating between adenoma and carcinoma.

Methods

Patients & Methods. We enrolled 43 patients (10 males and 33 females) from Ain Shams University Hospitals. The patients' ages ranged from 26-to-65. All human specimens were obtained with informed consent as approved by the Ain Shams University human research ethics committee. The patients were admitted for solitary thyroid nodules. They were subjected to surgeries that included total lobectomy and isthmectomy.

Pre-operative Assessment. A clinical examination of the thyroid nodule was performed. If a solitary thyroid nodule was suspicion to be malignant, it was excluded. Criteria for exclusion included hardness with limited mobility, lymphadenopathy, associated hoarseness, and dyspnea or dysphagia. The serum estimation of FT_3, FT_4, and ultrasensitive TSH were performed.

Radiological assessment included two procedures: an ultrasound of the thyroid gland using a real-time ultrasonographic scanner, and a thyroid scan using technetium 99m.

Intra-operative Specimens' Collection. Two samples from each patient was collected interoperatively using fine-needle aspiration biopsy. A sample was taken from the nodule and the other from neighboring thyroid tissue of the same lobe. Specimens were first preserved in formalin, then smeared onto slides, alcohol fixed, and then stained with haematoxlin and eosin. Specimens were evaluated by an expert pathologist.

The immunohistochemical staining SP method was performed to detect CD83. Formalin-fixed, paraffin-embedded thyroid specimens were washed three times in phosphate buffered saline. They were then treated with pepsin (0.5% in 0.01 N HCl) for 20 minutes at 37°C before staining for CD83. The specimens were then treated with normal goat serum for 20 minutes to block non-specific binding. The appropriate dilution (1:100) of mouse anti-human monoclonal antibody as primary antibody (PharMingen, San Diego, CA) was then added and incubated over night. The sections were then washed with phosphate buffered saline three times and reincubated with biotinylated goat anti-mouse immunoglobulin (1:200, DAKO, Denmark) at room temperature for 1 hour. After another wash with phosphate buffered saline, sections were soaked in alkaline phosphatese-conjugated streptavidin (DAKO), washed, and New Fuchshin (DAKO) were used as chromogen. Hematoxylin was used as a counter stain.

Expression of CD83 showing red granules was located on the membrane and/or in the cytoplasm of DCs; CD83-positive cells were counted over the whole specimens. The intensities of positive staining of S-100 was calculated by HpIAS1000 analysis system, shown as AGV (average grey value = |average positive grey value—average background grey value). The frequencies of CD83 positive cells were shown as the total numbers of cells/specimen.

Statistical Analyses. Data were collected, revised, verified, and then edited on a personal computer. Data were then analyzed statistically using SPSS statistical package version 13. The following tests were performed:

1. $\bar{x}$ = mean.
2. SD = standard deviation.
3. T-test for independent samples.
4. Paired sample t test.
5. ANOVA = analysis of variance.
6. Post hoc test to detect LSD.
7. Pearson correlation

Results

Figure 1 shows the percentage of pathological diagnosis of solitary thyroid nodules. A highly significant expression of mature dendritic cells CD83+ was found in papillary carcinoma as compared to normal thyroid ($p < 0.001$). There was also a significant difference between the CD83+ count in follicular carcinoma and follicular adenoma as compared to normal thyroid tissue ($p < 0.05$).

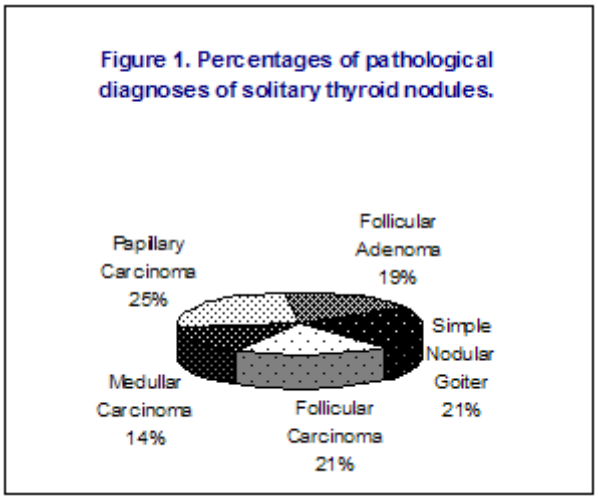


Figure 1. Percentages of pathological diagnoses of solitary thyroid nodules.

However, there was no significant difference ($p > 0.05$) between the CD83+ count in medullary carcinoma or simple nodular goiter of thyroid tissue and the CD83+ count in normal thyroid tissue taken from the same lobe (Table 1). A statistical significant difference ($p < 0.05$) was found between the CD83+ count in both follicular adenoma & carcinoma of thy-

Table 1. Expression of mature DC (CD83+) in solitary thyroid nodules and normal tissue.

Diagnosis	Number of samples taken from Solitary nodule		Number of samples taken from Normal tissue		*P* value
	Total number = 43		Total number = 43		
	Number of patients	*DC (CD83+)*	*Number of patients*	*DC (CD83+)*	
Papillary carcinoma	11	14.2873 ± 3.7656	11	0.2545 ± 0.5732	< 0.001
Medullary carcinoma	6	0.0233 ± 0.03726	6	0.0033 ± 0.00745	> 0.05
Follicular carcinoma	9	5.2144 ± 1.7162	9	0.03667 ± 0.0771	<0.05
Follicular adenoma	8	5.2200 ± 1.8386	8	0.3125 ± 0.4190	<0.05
Simple nodular goiter	9	0.11555 ± 0.1372	9	0.11555 ± 0.1372	> 0.05

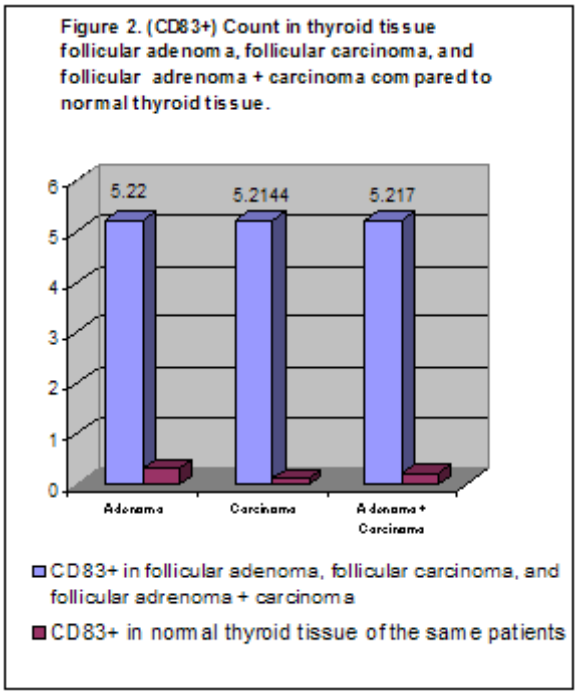
roid tissue and the CD83+ count in normal thyroid tissue of the same patients (Figure 2).

The mature DC expression in medullary carcinoma was very low and statistically significant compared to follicular carcinoma ($p < 0.05$). DC expression in medullary carcinoma was also significantly lower than that in papillary carcinomas ($p < 0.001$) (Table 2).

DC expression in papillary carcinoma was statistically higher ($P < 0.001$) than in follicular adenoma of thyroid tissue and simple nodular goiter of thyroid tissue (Table 3).

There was no significant correlation ($r = -0.146$) between different age groups and the CD83+ count. Also, in this study, no significance was found between DC expression and sex, family history, duration of the nodule, or the TSH level ($p > 0.05$)).

The sensitivity of dendritic cell expression for malignant thyroid carcinomas was 85%. The specificity was 82%.



Discussion

Thyroid cancer is a disease that may be fatal. This makes early diagnosis and treatment important in reducing mortality from the disease.

DCs have a central role in the initiation of primary immune responses. The cells are considered promising tools and targets for immunotherapy because of their capacity to stimulate naive T cells. DCs may play both prognostic and therapeutic roles in thyroid diseases.[4]

Histologically, normal thyroid tissue does not express DCs.[5] In this study, no mature DC expression was detected in normal thyroid tissue. In normal thyroid tissue, DCs are present as immature cells. They become mature only in response to a local inflammatory stimulus (the so-called danger signals), such as endotoxin (LPS), TNFa, and bacteria.[6]

It is clear from our study that mature dendritic cells were over expressed in papillary carcinoma ($\bar{x}$ of 14.2873) compared to normal thyroid tissue, other adenomas, and any other thyroid carcinoma. High

expression of DCs in papillary carcinoma can help differentiate between it and other papillary variants;

Table 2. Comparison between (CD83+) count in medullary carcinoma of thyroid tissue versus (CD83+) follicular carcinoma and papillary carcinoma of thyroid tissue.

DC(CD83+)	In thyroid medullary carcinoma		Mean difference	*P* value	Significance
	Mean ±SD	Number			
	0.0233 ± 0.03726	6			
In thyroid follicular carcinoma	5.2144 ± 1.7162	9	5.1911	< 0.05	Significant
In thyroid papillary carcinoma	14.2873 ± 3.7656	11	14.2639	< 0.01	Highly significant

this can be useful when fine needle aspiration is suspicious. Dendritic cells, then, may be a crucial diagnostic tool for papillary carcinoma.

The increased number of DCs in papillary carcinomas is possibly correlated with their good prognosis, irrespective of other morphological and clinical features.[7] Papillary cancers have also been shown to have a better cell-mediated immune response compared to controls and to follicular cancers. Thus the immune system, through the enhancement of inflammatory reactions induced by DCs, plays a major role in the clinical outcome of papillary thyroid cancer.[8]

There was no significant difference between mature DC expression in medullary carcinoma and normal thyroid tissue. This findings in our study agrees with the work of several other authors.[9,10,11]

Detecting DC expression can easily rule out or confirm medullary carcinoma. The reason is that compared to other carcinomas, DC expression in medullary carcinoma is significantly lower. DC immunotherapy has been introduced in the treatment of medullary thyroid carcinoma.[9]

In our study, positive risk factors such as a positive family history, age, gender, and the TSH level were not significantly related to DC expression. Our study results agree those of other studies concerning the lack of significant effect of positive risk factors in DC expression.[8,12] This indicates that DC expression is of significance in diagnosing papillary carcinoma irrespective of other factors.

It is established that FNAB is highly accurate in the diagnosis of thyroid malignancy. The sensitivity of the diagnostic procedure averages higher than 80% and specificity averages higher than 90%.[13] In our study, the sensitivity of the mature DC for malignant thyroid carcinomas was 85% and the specificity was 82%, for positive predictive value.

DC sensitivity for thyroid carcinoma will be 100% if medullary carcinoma is not included. The reason is that DC expression is positive for all thyroid carcinoma except for medullary carcinoma.

Conclusion

CD83+ DCs showed dense infiltration in papillary carcinoma. Its high density may be useful in the differential diagnosis of follicular carcinoma and other papillary variants. The very low DC expression in medullary carcinoma, in addition to fine needle aspiration biopsy, may help in the diagnosis of such cases. Dendritic cell expression potentially represents a new marker technique to improve sensitivity and specificity in evaluating solitary thyroid nodules.

References

1. Kaplan, M.M.: Clinical evaluation and management of solitary thyroid nodules. In *Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text*, 9th ed., edited by L.E. Braverman and R.D. Utiger. Philadelphia, Lippincott Williams & Wilkins, 2005, pp.996-1010.
2. Banchereau, J., Briere, F., Caux, C., et al.: Immunobiology of dendritic cells. *Annul. Rev. Immunol.*, 18:767-811, 2000.

3. Schott, M., Scherbaum, W.A., and Seissler, J.: Dendritic cell-based immunotherapy in thyroid malignancies. *Curr. Drug Targets Immune Endocr. Metabol. Disord*., 4(3):245-251,2004.
4. Ephrem, A., Bayry, J., Misra, N., et al.: Immunoglobulin-dependent regulation of dendritic cells in the context of autoimmune responses. *Transfus. Med. Hemother*., 32:369-372, 2005.
5. Bagriacik, E.U., Zhou, Q., Wang, H.C., et al.: Rapid and transient reduction in circulating thyroid hormones following systemic antigen priming: implications for functional collaboration between dendritic cells and thyroid. *Cell*, (15)212(2):92-100, 2001.
6. Steinman, R.M. and Pope, M.: Exploiting dendritic cells to improve vaccine efficacy. *J. Clin. Investig*., 109:1519-1526, 2002.
7. Batistatou, A., Zolota, V., and Scopa, C.D.: S-100 protein+ dendritic cells and CD34+ dendritic interstitial cells in thyroid lesions. *Endocr. Pathol. Summer*., 13(2):111-115, 2005.
8. Thompson, L.D., Wieneke, J.A., and Heffess, C.S.: Diffuse sclerosing variant of papillary thyroid carcinoma: a clinicopathologic and immunophenotypic analysis of 22 cases. *Endocr. Pathol*., 16(4):331-48, 2005.
9. Pfragner, R., Skofitsch, G., Hoger, H., et al.: Medullary thyroid carcinoma: autologous tumor cell lines for dendritic cell vaccination. *Clin. Cancer Res*., 10:2944-2953, 2005.
10. Scarpino, S., Stoppacciaro, A., and Ballerini, F.: Papillary carcinoma of the thyroid: hepatocyte growth factor (HGF) stimulates tumor cells to release chemokines active in recruiting dendritic cells. *Amer. J. Pathol*., 156(3): 831–837, 2000.
11. Kuwabara K, Nishishita T, Morishita M et al, Results of a phase I clinical study using dendritic cell vaccinations for thyroid cancer. *Thyroid*, 17(1):53-8, 2007.
12. Kabel, P.J., Voorbij, H.A.M., DeHaan, M., et al.: Intrathyroidal dendritic cells. *J. Clin. Endocrinol. Metab*., 66(1):199-207, 1988.
13. Dankle, S.K.: Clinical practice guidelines for the diagnosis and management of thyroid nodules. *Thyroid*, 23(2):303-37, 2002.