COMPARATIVE MORPHOMETRIC ANALYSIS OF THYROCYTES IN INVASIVE PART AND INTRANODULAR PART OF TUMOR IN MINIMALLY INVASIVE FOLLICULAR CARCINOMA OF THYROID

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ABSTRACT

• ***Objective:*** *Current study aims to compare the morphometric differences of capsule invading and non-invading thyrocytes in microinvasive follicular carcinomas of thyroid (MIFC).*

• ***Material and Method:*** *Materials of this retrospective study came from archival pathological materials cases of MIFC. Neoplastic thyrocytes in capsule invading and non invading areas were marked on hematoxylin and eosin stained sections. Nuclear morphometric features of thyrocytes from invading areas and non invading areas were obtained and compared.*

• ***Results:*** *A total of 13 pathological materials from 9 patients were selected. Capsule invading thyrocytes had significantly larger nuclear area and perimeter than those of non invasive thyrocytes in MIFC ($p<0.005$).*

• ***Conclusion:*** *Nuclei of capsule invading thyrocytes were larger than non invading thyrocytes. This difference has not been reported previously. This finding supports the proposal that follicular adenomas of thyroid may be the precursor lesion of MIFC in some cases.*

• ***Key Words:*** *Thyroid, minimally invasive follicular carcinoma, morphometry.* ***Nobel Med 2010; 6(3): 24-27***

TİROİDİN MİNİMAL İNVAZİV FOLLİKÜLER KARSİNOMLARINDA TÜMÖRÜN İNVAZİV KISMI İLE İNTRANODÜLER KISMINDAKİ TİROSİTLERİN KARŞILAŞTIRILMALI MORFOMETRİK ANALİZİ

ÖZET

• **Amaç:** Bu çalışmanın amacı minimal invaziv folliküler tiroid kanserlerinde kapsül invazyonu yapan ve yapmayan tirositler arasındaki morfometrik farkları incelemektir.

• **Materyal ve Metod:** Morfometri yardımıyla kapsül invazyonu yapan ve nodüler bölümde kapsül içinde kalan tirositlerin ölçümleri yapıldı.

• **Bulgular:** Dokuz hastaya ait tiroidektomi materyalinden elde edilen kesin olarak kapsül invazyonu izlenen 13 patolojik materyal incelendi.

Sonuçlarımız kapsül invazyonu yapan tirositlerin non invaziv tirositlere göre nükleer alanlarının ve çaplarının daha büyük olduğunu gösterdi. Bu fark istatistiki olarak da anlamlıydı ($p<0,005$)

• **Sonuç:** İnvaziv nitelikteki neoplastik tirositlerin nükleer alanları daha büyüktü. Bu bulgu literatürde daha önce bildirilmemiştir. Bu sonuç soliter folliküler adenomların prekürsor lezyon olabileceğini öne süren görüşleri desteklemektedir.

• **Anahtar Kelimeler:** .Tiroid, minimal invaziv folliküler karsinoma, morfometri **Nobel Med 2010; 6(3): 24-27**

INTRODUCTION

Follicular lesions of thyroid contains a variety of thyrocyte proliferations. These proliferations include common follicular nodules, follicular adenomas, follicular carcinomas and other tumors with follicular pattern. Follicular adenomas (FA) are benign, encapsulated tumors that are more prevalent than follicular carcinomas[1] FA are regarded as true neoplasms, since several studies have detected their clonal nature.[2,3] From the pathological and surgical point of view, the term FA should reserved for lesions that are solitary and have a conspicous fibrous capsule. Follicular carcinoma is less prevalent than FA and constitutes about 5% of all thyroid cancers;[1-3] Follicular carcinoma is usually presented in two distinct types of invasion i.e. widely invasive and minimally invasive. In contrast to widely invasive follicular carcinomas, minimally invasive follicular carcinomas (MIFC) are well-differentiated tumors associated with an excellent prognosis. Metastatic diseases infrequently reported in MIFC and ten year overall survival is greater than 95% [4-6] The diagnosis of minimally invasive follicular carcinoma using World Health Organization criteria is based upon the presence of transcapsular and/or vascular invasion in resected specimens.[1,6] The aim of this study was to investigate the nuclear morphometric profiles of neoplastic thyrocytes of MIFC and to make possible statistical comparison between the thyrocytes inside the nodule and invasive areas.

MATERIAL and METHOD

Case selection and histological assessment The archival material (paraffin blocks and Hematoxylin eosin stained sections) of the cases that were diagnosed as follicular carcinoma of thyroid were retrieved from archives. All histological material were reassessed for the confirmation of diagnosis according to previously published criteria. For this particular study only informative MIFC cases that have unequivocal capsular invasion were included. Other types of thyroid carcinomas such as anaplastic carcinoma, papillary carcinoma, and follicular variant of papillary carcinoma were excluded. Four micron thick sections were recut from paraffin blocks and stained with hematoxylin and eosin at the same session. During the histological assessment of cases, the capsular penetration which often has a mushrooming appearance has been marked on the glass slide (Figure).

Nuclear morphometry

Nuclear morphometry was performed on the representative H&E stained slides that contains the capsular penetration with optimum histologic detail. The Kontron Electronik imaging system, comprising

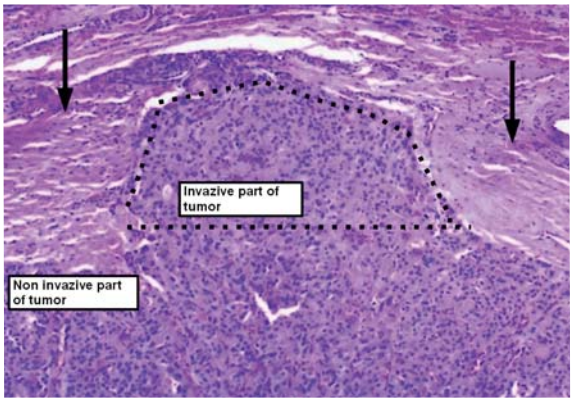

Figure. Topographic microanatomy of microinvasive follicular carcinoma of thyroid (MIFC). Arrows show the fibrous capsule that encircle the lesion. In dotted area, neoplastic thyrocytes ruptured the fibrotic capsule of lesion and grew outward in volcano shaped fashion. This microscopic finding is the sine qua non for pathological diagnosis of MIFC. Current study compares the morphometric features of cells in invasive area with noninvasive area.

a light microscope (Axiophot 2, Zeiss) with a Sony CCD camera linked to a computer using KS400 Release 2.0 software, was used. For each case, 150 thyrocytes epithelial nuclei were randomly selected from invasive areas at a magnification of 400X. The images were then digitized and the nuclei outlined using a mouse attached to the computer. As contol group, the thyrocyte nuclei on the areas beneath the capsule were also measured. Morphometric parameters that have been assessed were nuclear area and perimeter and feret circle and feret ratio. The feret ratio is a ratio of minimum to maximum feret diameter, with a maximum value of 1 corresponding to a circle. Feret circle, a shape factor, is described by the formula $(4\pi \text{ area})/\text{perimeter}^2$ area. Both feret circle and feret ratio are measures of ellipticity. These values of were generated by the computer.

Statistical analysis

Statistical analysis was performed using the statistical software Stata. Nonparametric tests were to compare the groups for significant differences. A statistically significant result was defined when a P value is above <0.005.

RESULTS

We studied thirteen pathological materials that have been obtained from nine patients who were undergone lobectomy or total or subtotal thyroidectomy. All cases were diagnosed as microinvasive follicular carcinoma of thyrodi. Patient age ranged from 34 to 67 years , and the male-to female ratio was 1:2 (3 males, 6 females). Tumor size of cases ranged from 0.5 to 3cm, with a median of 1.5 cm. None of the patients have regional or distant metastasis. In all cases the capsular penetration of follicular neoplasm which has mushroom like appearance was conspicously→

Table 1: Descriptive statistics of invasive thyrocytes and non invasive thyrocytes.

	Invading	Noninvading
Mean	69,0978	65,1886
Std. Error of Mean	0,54767	0,51799
Median	65,8275	61,8942
Std. Deviation	20,67407	19,88047
Variance	427,41703	395,23326
Skewness	1,259	1,255
Std. Error Skewness	0,065	0,064
Kurtosis	2,494	3,419
Std. Error of Kurtosis	0,130	0,127
Range	147,71	26,90
N	1.425	1.473

Table 2: Statistical analysis

	Perimeter	Area
Mann-Wihitney U	932.434.500	932.589.000
Wilcoxon W	2.018.035.500	2.018.190.000
Z	-5,200	-5,192
Asymp. Sig. (2-tailed)	,000	,000

observed. Vascular invasion was not detected in any case. Surgical margins were free of tumor in all cases. Descriptive statistics of nuclear area are summarized in Table 1.

Statistical analysis (Table 2) showed that nuclear area and perimeter of capsule penetrating thyrocytes are significantly larger than control group (Mann Whitney U test p=0.000).

DISCUSSION

Extensive amount of research has been done into the application of morphometric image analysis in neoplasms of the thyroid.[7-11] Some researchers have tried to establish nuclear morphometry by computerized image analysis as an additional tool in the differential diagnosis of thyroid follicular neoplasms especially in patients with solitary thyroid nodules.[9,10] Although the results of these studies are inconclusive and equivocal, most observed that computer assisted image analysis may be an adjunct to the clinical evaluation in the assessment of thyroid follicular neoplasms. Accurate distinction of follicular adenoma from follicular carcinoma probably poses maximum difficulty in surgically removed thyroid specimens as well as in preoperative fine needle aspiration cytology samples. In resected specimens the only available means are histologic demonstration of unequivocal vascular or capsular invasion. For classifying a MIFC, the tumor must penetrate through the entire thickness of the capsule to be regarded as having transcapsular invasion. When the follicular carcinoma penetrates through the capsule, it often has a mushrooming appearance. In many cases, the extracapsular tumor forms a new fibrous capsule along its leading edge, which borders on the normal thyroid parenchyma. In an analysis of observer variations for histologic diagnosis of thyroid cancer, follicular carcinoma was the most common diverging diagnosis where the final diagnosis was a benign lesion.[12,13] In addition the lack of universally accepted cytologic parameters of distinction between follicular adenoma and follicular carcinoma is the main reason for failure of minimally invasive investigative procedure of fine needle aspiration (FNAC) to offer further diagnostic direction for follicular neoplasm group of lesions. In our study we were able to show that nuclear morphometric parameters of capsule penetrating thyrocytes are different. This observation may provide a valuable parameter in morphometric analysis of the future studies for distinguishing the FA from FCA.

The pattern specific nuclear morphometric changes of thyroid lesions have been addressed in a few studies.[14,15] Nuclear morphometry has been utilized to distuingish the papillary structures of benign versus malignant origin and significant discrimination between follicular variant of papillary carcinoma and the broad group of follicular neoplasms.[15] To our best knowledge, the morphometric analysis of capsule invasion was not separately studied previously.

One limitation of current study is that measurements were recorded as pixels not in metric units. Since we utilized archival pathological material, fixation time, fixation solutions, methods as well as processing techniques were different in each case. These non uniform preparation contribute to the unforeseen artifactual changes in nuclear dimensions of cells. So we decided not to record metric units of each measurement, instead we prefered to compare the nuclear features of cells on the same pathological material. Hence we believed we avoided the problems associated with different fixation methods and processing techniques.

Whether or not some solitary follicular nodules have the biologic potential to become carcinoma is unknown; the findings of aneuploid cell populations in 27% of such lesions suggest that some of these may represent carcinoma in situ.[16] Our observation in the current→

study may serve as morphometric evidence that supports previously proposed hypothesis that follicular adenomas was thought to be preceded follicular carcinomas because of similarity of carcinogenetic features.[16-18] In the development of follicular adenoma from normal thyrocytes PAX8-PPARY fusion protein may play an important role. This protein is a product of chrosomal transformation that is t (2; 3) (q13; P25). In addition to q13: p25 transformation recent cytogentical abnormalitis have also been identified in follicular tumors.[19-22]

CONCLUSION

The main purpose of this study was to compare the morphometric differences of thyrocytes that are located in invasive areas with non invasive areas. Our results showed that nuclei of capsule penetrating thyrocytes are larger from the those of thyrocytes in noninvasive areas. This result support the proposal that follicular adenomas might be the precursor lesions of MIFC in some cases.

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✔ DELIVERING DATE: 04 / 07 / 2009 • ACCEPTED DATE: 04 / 02 / 2010

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