The value of rapid semi-automated morphometric analysis system in predicting malignancy in thyroid follicular neoplasm

Thair Wali Ali*
***College of Medicine,University of Al-Qadisiyah**
E-mail: thairsudani1977@gmail.com; thair.ali@qu.edu.iq

Abstract
Aims: To constuct and evaluate a rapid semiautomated system in discriminating malignant from benign follicular thyroid neoplasms.
Methods: Sixty formalin fixed paraffin embeded blocks of follicular thyroid neoplasms were retreived and slides were prepared and H and E stained. A morphometric system was constructed to be as rapid as possible and was tested.
Results: No signficant difference was found in ratio of largest nuclear to smallest nuclear diameter between follicualr adenoma and follicualr carcinoma groups.
Conclusion: Rapid semiautomated morphometric analysis is of no help in predicting malignancy in follicular thyroid neoplasm.
Key words: morphometry, thyroid, carcinoma

Introduction

Follicular adenoma and follicular carcinoma of the thyroid gland are tumors of follicular cell differentiation. In two autopsy series, the incidence of thyroid adenoma was 3 and 4.3%. The ratio of follicular adenoma to follicular carcinoma in surgical specimen is approximately 5 to 1.[1].

Follicular carcinoma has microscopic features that are similar to a follicular adenoma. However, a follicular carcinoma tends to be more cellular with a thick irregular capsule, and often with areas of necrosis and more frequent mitoses. A follicular carcinoma cannot be distinguished from a follicular adenoma based on cytologic features alone. It is distinguished from a follicular adenoma on the basis of capsular invasion, vascular invasion, extrathyroidal tumor extension, lymph node metastases, or systemic metastases.[2, 3] However, evaluation of these features can be challenging on histologic examination due to the presence of incomplete capsular penetration or equivocal vascular invasion, and for this reason, many end up with a general inconclusive diagnosis of "follicular lesion".[4]

Immunohistochmeical markers have been extensively used in published researches all over the world for the purpose of discrimination between malignant and benign follicular thyroid neoplasma; however the results were conflicting. Several immunohistochemical markers have been tested including: TFF3, galectin-3, HBME-1, CK19, EMMPRIN, and GADD153, and in all situations more than one marker was tested and the results were inconclusive unless combined with morphologic features. In addition the sensitivity and specificity of these immunohistochemical markers were not always convincing.[5-7]

Genetic markers have also been tested and the list included: ELMO1, EMCN, ITIH5, KCNAB1, SLCO2A1, RAS, BRAF, PAX8/PPARγ and RET/PTC and so much many else;[8-14] however the results carried enough controversy to permit reproduction of the tests in further other reaserches that added nothing more than the early established idea that no one marker can replace pathologist eye in diagnosing malignant thyroid follicular neoplasm.

Materials and methods

Paraffin blocks

Sixty paraffin blocks of thyroid follicular neoplasms, 30 follicular adenoma cases and 30 follicular carcinoma cases, were retreived from archival materials stored in the teaching laboratories of Gaghdad Medical city, Al-Imamain Al-Kadhymain medical city and Al-Dewaniyah teaching hospital. From each block one 5 µm thin section was obtained and stained by hematoxilin and eosin (H and E) routine stain accoridng to the routine protocol adopted by teaching laboratory in Al-Dewaniyah teaching hospital.

Morphometric analysis

For the purpose of construction of a rapid semiautomated morphometric analysis, the following steps were carried out: A light microscope (Leika DM 2500, Germany) was used to capture representative immages. Each slid was serched for a represantative field and then a single image was taken at 40X power. All immages were transferred then into a laptop personal computer (Fujitsu, Japan). Analysis of immages was carried out using paint software which is already installed in the accessory package of Windows 7 as shown in figures 1, 2, 3 and 4.

The first step was to choose a single nucleus in the upper right field with most oval shape. The second step was to measure its largest diameter and for this purpose the ruler option in the paint software was made active. Then the pointer was moved to touch the most outer border of one of the poles of the nuclus (yellow arrow in figure 1). The coordinates were shown by the paint software in the lower left corner (blue arrow in figure 1). These coordinates were considered (X_1, Y_1) for the largest diameter.

Using the same procedure (X_2, Y_2) coordinates for the largest diamter were obtained (figure 2). To measure the largest diameter passing in the center of the nuclus, the following simple mathematical formula was used formula [Largest diameter = square root of the sum of $(X_2-X_1)^2 + (Y_1-Y_2)^2$], and the use of Microsoft Office Excel 2007 to construct this mathematical formula, as shown in figure 5.

Then the (X_1, Y_1) and (X_2, Y_2) coordinates for the smallest diameter were obtained (figure 3 and 4), but here the pointer was made to touch the two points that identify the narrowest diameter passing in the center of the nucleus. The same mathematical formula was applied and the smallest diameter was calculated, then the ratio of (Largest diameter/smallest diameter) was calculated also using Microsoft Office Excel 2007.

This process was repeated five times, since 5 nuclie for each case was included, one in the right upper field, the second in the left upper field, the third in the left lower field, the fourth in the right lower field and the fifth in the center of the field.

The image presented in figures 1 through 4 for purpse of illustration was retreived from (http://www.pathologyoutlines.com/topic/thyroidfollicular.html).

Statistical analysis

Statistical analysis for data of the current study were analysed using SPSS version 16 and Microsoft Office Excel 2007. Numeric variables were tested for normality distribution using Shapiro-Wilk tests. Mann Whitney U test was used to cmpare mean ratio of largest to smallest diameter between follicular carcinoma dn follicualr adenoma groups. The level of signficance was considered at p-value of less than or equal to 0.05.

Figure 1: Identification of (X_1,Y_1) for the largest diameter. Yellow arrow indicates the site of pointer and the blue arrow indicates the site of numeric coordinates

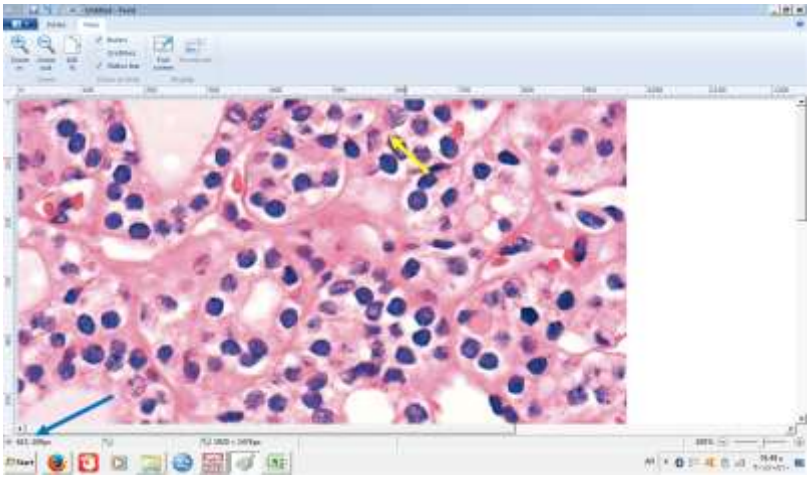


Figure 2: Identification of (X_2,Y_2) for the largest diameter. Yellow arrow indicates the site of pointer and the blue arrow indicates the site of numeric coordinates

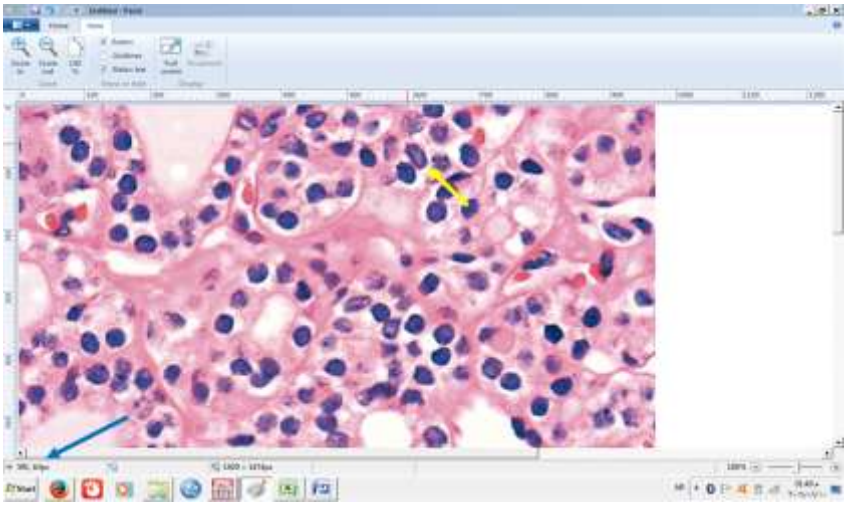


Figure 3: Identification of (X_1,Y_1) for the smallest diameter. Yellow arrow indicates the site of pointer and the blue arrow indicates the site of numeric coordinates

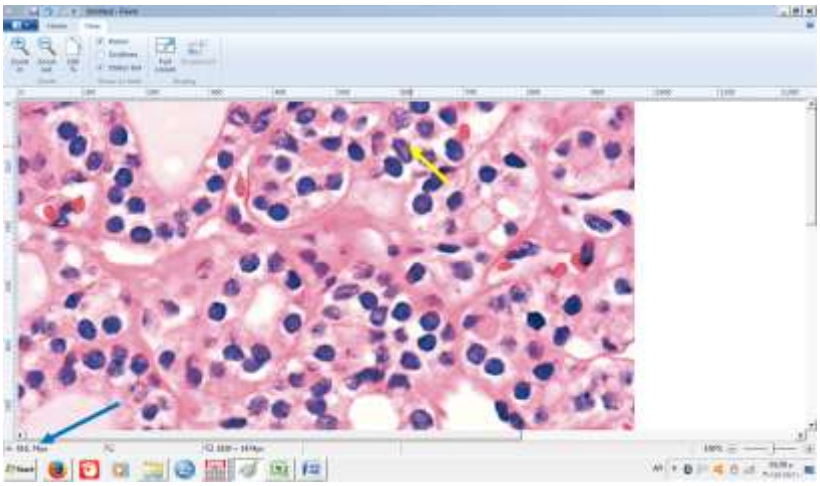


Figure 4: Identification of (X_2,Y_2) for the smallest diameter. Yellow arrow indicates the site of pointer and the blue arrow indicates the site of numeric coordinates

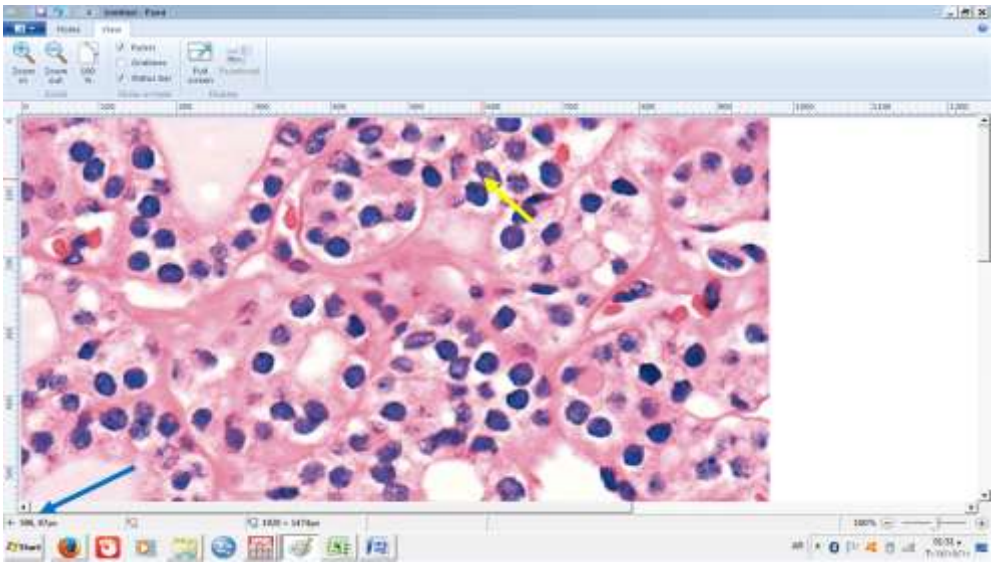
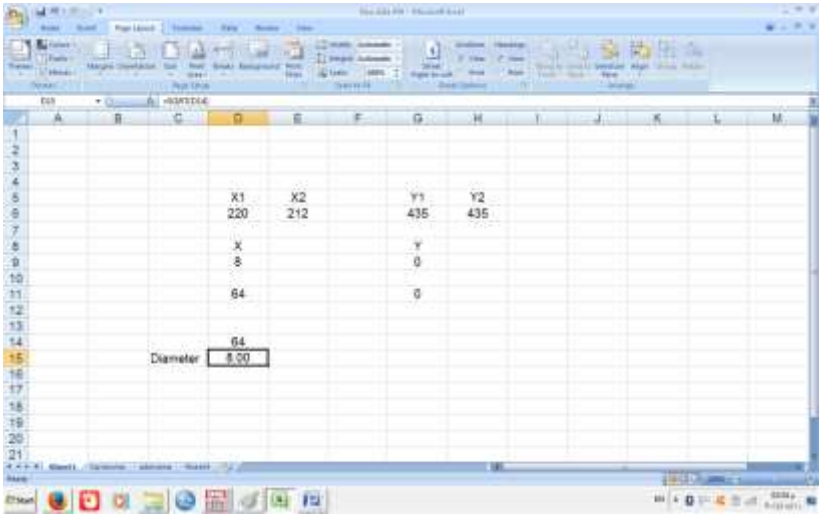


Figure 5: Setting the function for calculation of the diameter using Microsoft Office Excel 2007



Results

The numeric variable (ratio) which represents the ratio of largest to smallest diameter was introduced into an SPSS spread sheet. This variable was tested for normality and descriptive statistics were presented in table 1. This variable (ratio) did not follow a normal distribution since there was a significant deviation from normality distribution in follicular carcinoma and follicular adenoma groups, as shown in table 1 and figure 6. There was insignificant difference in mean ratio between follicular carcinoma and follicular adenoma groups (P>0.05) (figure 7).

Table 1: Statistical analysis of ratio variable in follicular carcinoma and follicular adenoma group

Statistic	Follicular carcinoma (n = 30)	Follicular adenoma (n = 30)
Mean	2.47	2.29
95% Confidence Interval for Mean	2.23 - 2.72	2.16 - 2.42
Standard deviation	0.66	0.34
Minimum	1.53	1.82
Maximum	4.45	3.25
Median	2.32	2.25
Interquartile Range	1.05	0.49
Skewness	0.92	0.92
Kurtosis	1.15	0.68
Shapiro-Wilk test	<0.05	<0.05

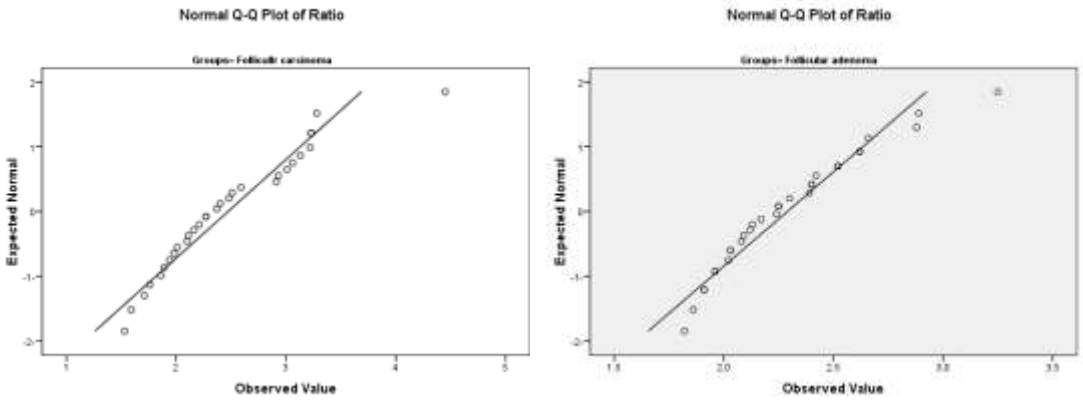


Figure 6: Normal Q-Q plot of ratio variable in follicular carcinoma and follicular adenoma groups

Discussion

The presence of enough controversy in published literatures about the role of morphometric analysis in discriminating between malignant and benign thyroid follicular neoplasms was the motive behind conduction of the present study.

In the current study a trial of constructing a simple rapid semiautomated morphometric analysis system was established. Most of the published literatures described complex systems of fully or semiautomated morphometric analysis that are time consuming, coasty and laborious enough to caused fatigue to the examining pathologist. However, this rapid method proved to be of no value in predicting malignant behavior in thyroid follicular neoplasms.

Kang *et al*., utilized the digital image analysis using ImagePro 6 software (Media Cybernetics, Bethesda, MD, USA) and used two variables, area and perimeter, to compare suspicious lesions with benign and reactive lesions; however the results were not 100% discriminating benign from other lesions. [15]

Wang *et al*. carried out a complex digital morphometric analysis aiming at differentiation between malignant and benign follicular thyroid neoplasms. Although they described significant correlation between morphometric variables and malignant behaviuor; the method was complicated enough to be difficult to use in routine clinical practice. [16]

Several authors tested a lot of complex systems of digital analysis to differentiate between malignant and benign follicular thyroid lesions both on histological sections and cytological preparations. [17-20] Beside the laborious effort needed to carry out the described image analysis in these literatures, the results were not 100% confident in establishing clear cut discrimination between benign and malignant lesions.

Accordingly, the routine establishment of vascular and capsular invasion in paraffin embedded formalin fixed tissue sections that are stained with H and E stain remain the gold standard and the most reliable method in clinical practice to diagnose malignant follicular thyroid neoplasm with in an acceptable accuracy.

In conclusion, rapid semiautomated morphometric analysis is of no help in predicting malignancy in follicular thyroid neoplasm and that routine histological examination of paraffin embedded formalin fixed tissue section by well trained pathologist is the optimum method to diagnose thyroid follicular carcinoma.

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