Role of morphometry in the cytological differentiation of benign and malignant thyroid lesions

ABSTRACT

Context: Thyroid nodules represent a common problem, with an estimated prevalence of 4–7%. Although fine needle aspiration cytology (FNAC) has been accepted as a first line diagnostic test, the rate of false negative reports of malignancy is still high. Nuclear morphometry is the measurement of nuclear parameters by image analysis. Image analysis can merge the advantages of morphologic interpretation with those of quantitative data.

Aims: To evaluate the nuclear morphometric parameters in fine needle aspirates of thyroid lesions and to study its role in differentiating benign from malignant thyroid lesions.

Material and Methods: The study included 19 benign and 16 malignant thyroid lesions. Image analysis was performed on Giemsa-stained FNAC slides by Nikon NIS-Elements Advanced Research software (Version 4.00). Nuclear morphometric parameters analyzed included nuclear size, shape, texture, and density parameters.

Statistical Analysis: Normally distributed continuous variables were compared using the unpaired t-test for two groups and analysis of variance was used for three or more groups. Tukey or Tamhane’s T2 multiple comparison test was used to assess the differences between the individual groups. Categorical variables were analyzed using the chi square test.

Results and Conclusion: Five out of the six nuclear size parameters as well as all the texture and density parameters studied were significant in distinguishing between benign and malignant thyroid lesions ($P < 0.05$). Cut-off values were derived to differentiate between benign and malignant cases.

Key words: Fine needle aspiration cytology (FNAC); image analysis; nuclear morphometry; thyroid lesions

Introduction

Thyroid nodules represent a common problem, with an estimated prevalence of 4–7% in the adult population for palpable nodules.[1] In India, the prevalence of a palpable thyroid nodule is approximately 12.2%, according to a recent study.[2] Although thyroid cancer is quite rare with an incidence of 8.7 per 100,000 people per year, this seems to be increasing over the years.[3] As for most head and neck cancers, early stage diagnosis and management lead to better outcomes. Fine needle aspiration cytology (FNAC) of the thyroid gland is now a well established first line diagnostic test for the evaluation of diffuse thyroid lesions as well as nodular lesions of thyroid, with the main purpose of confirming benign lesions and thereby reducing unnecessary surgery.[4] Thyroid FNAC has been estimated to have a sensitivity for malignancy between 65 and 98% and specificity between 72 and 100%.[5] The rate of false negative reports is still high, and

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How to cite this article: Khatri P, Choudhury M, Jain M, Thomas S. Role of morphometry in the cytological differentiation of benign and malignant thyroid lesions. J Cytol 2017;34:1-4.
hence, there is a need for introducing additional methods to improve the accuracy of cytological diagnosis. Some of the sophisticated and newly developed tools, such as flow cytometry and microarray technology, lack accompanying morphologic information. Image analysis is a method that may merge the advantages of morphologic interpretation with those of quantitative data. Morphometry is the quantitative analysis of geometric features of structures with any dimension. The size, shape, and chromatin pattern of nuclei, defined as nuclear morphometry of malignant cells, are found to be different from nonmalignant cell nuclei, which enables nuclear morphometry to distinguish between benign and malignant cases. Using nuclear morphometry, we can quantify a number of parameters such as those related to nuclear size and shape. It has been suggested that nuclear morphometric parameters such as nuclear area and perimeter, and nuclear area coefficient of variation, and shape factors may allow differentiation between thyroid lesions. Application of morphometry to cytology would be useful for the purpose of automated screening as well as accurate diagnosis of thyroid tumors. Till now, majority of image analysis studies have been performed on histological sections. Hence, the purpose of this study is to evaluate the significance of nuclear morphometry in cytological aspirates of thyroid masses.

Material and Methods

A total of 35 cases (19 benign and 16 malignant cases) were included in the study. These included multinodular goitre (17 cases), follicular neoplasm (5 cases), follicular variant of papillary carcinoma (FVPC) (2 cases), papillary carcinoma (5 cases), and medullary carcinoma (6 cases). All cases were confirmed on histopathology. Out of 5 follicular neoplasms diagnosed on cytology, 2 were diagnosed as benign (follicular adenoma) and 3 were diagnosed as malignant (follicular carcinoma) on histopathology. Mean age of presentation of benign cases was 37.2 years (range: 19–60 years) while that of the malignant cases was 47.84 years (range: 25–58 years). FNA smears were air-dried and stained with Giemsa stain. Slides with well preserved, and evenly spread FNAC material were used for morphometry. The representative images were captured with a digital camera (Nikon DS-Ri 1-U3,Tokyo,Japan.Camera control unit DS-U3) at 1000× for all the cases. The saved images were opened in Nikon Imaging Software-AR (version 4.0), and 50 nonoverlapping nuclei were selected and outlined using the mouse of the computer at the same zoom settings [Figure 1]. All measurements were tabulated in Excel sheets. Nuclear morphometric parameters analyzed were the nuclear size parameters (nuclear area, equivalent diameter, maximum feret, minimum feret, perimeter and coefficient of variation of nuclear area or NACV), nuclear shape parameters (shape factor and largest to smallest diameter ratio or l/s ratio), nuclear texture parameters (sum and mean intensity, sum and mean brightness), and nuclear density parameters (sum and mean density). NACV was calculated by (standard deviation of nuclear area/mean nuclear area) × 100. It is a measure of variation in the nuclear area in a given smear. Normally distributed continuous variables were compared using the unpaired t-test for two groups and analysis of variance was used for three or more groups. Tukey or Tamhane’s T2 multiple comparison test was used to assess the differences between the individual groups. Categorical variables were analyzed using the chi square test. For all statistical tests, a $P$ value of less than 0.05 was taken to indicate a significant difference. A receiver operating characteristics (ROC) analysis was calculated to determine optimal cut-off values for nuclear area, equivalent diameter, maximum feret, minimum feret, and nuclear perimeter. The area under the curve (AUC), the sensitivity, and the specificity was calculated to analyze the diagnostic value of all these markers.

Results

Five of the nuclear size parameters (nuclear area, equivalent diameter, maximum feret, minimum feret, and perimeter) had lower mean values in benign cases (31.80 µm², 6.29 µm, 7.13 µm, 5.84 µm, and 20.52 µm, respectively) than malignant cases (44.41 µm², 7.44 µm, 8.41 µm, 6.91 µm and 24.20 µm, respectively). All these nuclear size parameters were highly significant in differentiating between benign and malignant
cases ($P < 0.005$). NACV (coefficient of variation of nuclear area) was not significant in distinguishing between benign and malignant thyroid lesions. None of the nuclear shape parameters were significant in distinguishing between benign and malignant thyroid lesions. Among the nuclear texture parameters, mean values of mean intensity and sum intensity were lower in benign cases than in malignant cases and mean values of mean brightness and sum brightness were lower in benign cases than in malignant cases. All these were significant in distinguishing between benign and malignant thyroid lesions ($P < 0.05$). Both sum density and mean density values were lower in benign cases than malignant cases and were significant in differentiating between benign and malignant cases ($P < 0.05$). Cut-off values between benign and malignant groups for mean nuclear area, equivalent diameter, maximum feret, minimum feret and perimeter were 39.5 $\mu$m$^2$, 7.10 $\mu$m, 7.9 $\mu$m, 6.0 $\mu$m, and 23.0 $\mu$m, respectively, with sensitivity of 75, 75, 81.3, 100, and 68.4%, respectively, specificity of 89.5, 89.5, 89.5, 57.9, and 94.7%, respectively, and excellent accuracy (AUC of 0.878, 0.875, 0.895, 0.842, and 0.878, respectively). Cases with values below these cut-offs were considered to be benign and those with values above these cut-offs as malignant.

**Discussion**

Change in nuclear morphology is the hallmark of cancer diagnosis. Features of the nucleus indicating malignancy are enlarged nuclei, hyperchromasia, coarse chromatin, and prominent nucleoli. In our study, five of the nuclear size parameters (nuclear area, equivalent diameter, maximum feret, minimum feret and perimeter) had lower mean values in benign cases than malignant cases and the differences were significant ($P < 0.005$). Giobanu *et al.*[14] also found that the nuclear area and mean nuclear diameter in case of follicular carcinoma was significantly larger than benign cases (which included follicular adenoma, nodular goitre, and lymphocytic thyroiditis). Similarly, Tseleni-Balafouta *et al.*[15] calculated the mean nuclear area and mean major nuclear axis to be larger in follicular carcinoma than that in follicular adenoma and hyperplastic nodule. Rout *et al.*[16] found similar results with nuclear area and mean nuclear diameter to be highest in papillary carcinoma than that in follicular carcinoma and follicular adenoma and least in nodular colloid goitre. Wright *et al.*[17] also found that the mean nuclear area and mean nuclear perimeter in Giemsa-stained smears of papillary carcinoma and follicular carcinoma were significantly larger than follicular adenoma and multinodular goitre (MNG).

Cut-off values between benign and malignant groups for mean nuclear area, equivalent diameter, maximum feret, minimum feret, and perimeter were 39.5 $\mu$m$^2$, 7.10 $\mu$m, 7.9 $\mu$m, 6.0 $\mu$m, and 23.0 $\mu$m, respectively. There are differences in the observed values of different morphometric features of the present study as compared to other studies due to various analytical factors (patient ethnicity, slide preparation, staining differences, difference in software used for morphometry) in different studies. The cut-off values of nuclear size parameters derived from the present study were not comparable to other studies. Thus, we concluded that nuclear morphometry can help in differentiating between benign and malignant thyroid lesions, however, there is a need to carry out further studies on a larger group of cases to evaluate its usefulness in borderline cases.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

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