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ORIGINAL ARTICLE

Nuclear morphometry and texture analysis on cytological smears of thyroid neoplasms: a study of 50 cases

Lopamudra DEKA *MD*, Shilpa GUPTA *MD*, Ruchika GUPTA *MD**, Kusum GUPTA *MD*, CJ KAUR *MD* and Sompal SINGH *MD*

*Departments of Pathology, Hindu Rao Hospital and *National Institute of Cancer Prevention & Research, Noida, India*

Abstract

Background: Fine needle aspiration cytology (FNAC) is a reliable and reproducible diagnostic technique for thyroid lesions with certain limitations. Computed morphometric methods have been introduced with a view to improve the diagnostic yield of thyroid aspirates. However, a review of the existing literature revealed conflicting reports regarding morphometric parameters in thyroid neoplasms. *Materials and Methods:* This study included 50 cases of thyroid lesions (20 cases of colloid goitre, 15 of follicular adenoma, 5 of follicular carcinoma and 10 papillary carcinomas). Digital images of cytologic smears of these cases were captured using a dedicated photomicrography system and nuclear profiles traced manually. With self-designed image analysis software, nuclear morphometric measurements, including texture analysis, were performed. Discriminant analysis was performed including the morphometric parameters and percentage of correctly classified nuclei noted. *Results:* Nuclear morphometry parameters showed that papillary thyroid carcinoma had the highest perimeter, area, radius and elongation factor compared to other thyroid lesions. Discriminant analysis revealed that altogether 77.9% of cells could be correctly classified to their lesion category based on the nuclear morphometric and textural parameters. Of the neoplastic cases, 84.5% of cells of follicular neoplasms and 72.5% of papillary carcinoma were classified to the respective category. *Conclusion:* Nuclear morphometry, including texture analysis, can assist in the cytologic diagnosis of thyroid lesions, considering the high degree of accuracy of classification. Further studies and methodological refinements can achieve higher accuracy.

Keywords: morphometry, texture analysis, thyroid, aspiration cytology

INTRODUCTION

Fine needle aspiration cytology (FNAC) is a favoured minimally invasive technique for pre-operative diagnosis of nodular lesions of the thyroid.[1] The utility of FNAC is, however, limited by the problems in differentiation of follicular adenoma and goitre from follicular carcinoma.[2] Few earlier authors have shown an improvement in FNAC diagnosis using morphometric analysis[3,4] but others have failed to confirm these findings.[5] Wright *et al*, in their study, showed significant difference in nuclear area and perimeter between benign and malignant thyroid lesions. However, wide variation in values precluded routine use of this technique.[4] Karslioglu *et al* evaluated multiple nuclear morphometric parameters with discriminant analysis and found that 58.8% of cases could be correctly classified as nodular goitre or follicular lesion.[6]

Nuclear textural parameters have been studied very rarely in few studies. A study by Murata *et al* using morphological abstraction method showed that papillary carcinoma had larger, irregular nuclei with higher contrast of chromatin pattern while follicular carcinoma showed larger nuclei with monotonous chromatin pattern.[7]

The present study was aimed at evaluating nuclear morphometric and textural parameters in various thyroid lesions on aspiration cytology.

MATERIALS AND METHODS

This study included 50 cases of histopathologically confirmed thyroid lesions diagnosed over a three-year period (2007-2009). These included

Address for correspondence: Dr Sompal Singh, Specialist, Department of Pathology, Hindu Rao Hospital, Malka Ganj, Delhi - 110007, India. Tel: +91-11-23919613. Email: sompal151074@gmail.com

20 colloid goitre and 30 neoplastic lesions comprised of follicular adenoma (15 cases), follicular carcinoma (5 cases) and papillary carcinoma (10 cases).

All these patients had been subjected to FNAC on an out-patient basis. The FNA smears were air-dried and stained with Giemsa stain. Although nuclear features and texture are better appreciated on Papanicolaou stained smears, however, in our department, Giemsa stain was the conventionally used stain. Based on the cytological impression, patients underwent surgery varying from lobectomy to total or subtotal thyroidectomy. The resected specimens were submitted for histopathologic examination. The histologic diagnoses were reviewed by four histopathologists (RG, KG, CJK, SS) and a consensus diagnosis reached.

Morphometric study

For morphometric studies, the FNAC smears were re-examined using a standard microscope with dedicated photomicrography attachment. Areas with higher cellularity, non-overlapping nuclei and no/minimal obscuration by blood were selected for analysis. A minimum of three slides were photographed for each case. Digital images were captured using 40X objective and stored in TIFF format. Nuclear profiles were traced manually in the image analysis software using a pen-tablet device. A minimum of 100 non-overlapping nuclei were traced in each case. Morphometric measurements were obtained using image analysis software written by one of the authors (SS). The parameters included nuclear morphometry (perimeter, area, major axis, minor axis, shape factor, compactness, circularity, elongation) as well as textural parameters analyzed by first order statistics, i.e. mean and standard deviation and second order statistical measures using co-occurrence matrix. In order to achieve uniformity in the nuclear analysis, the nuclear features and texture were standardized in each slide against the nucleus of a small lymphocyte present in the smear. In each case, the morphometric and textural analysis took about half a minute by the software. The manual nuclear tracing took approximately 2-3 minutes per image.

The cases were categorized as colloid goitre, follicular neoplasms (including both adenoma and carcinoma) and papillary carcinoma, based on the final histopathologic examination. Discriminant analysis was performed with the nuclear morphometric and textural parameters using statistical software. The percentage of correctly classified nuclei was recorded. In addition, the percentage of cases categorized accurately was also calculated after discriminant analysis.

RESULTS

The cases comprised 20 colloid goitre, 20 follicular neoplasm and 10 papillary carcinoma (Fig. 1). The salient morphometric parameters in the three groups of cases are summarised in Table 1. Since the numbers of follicular

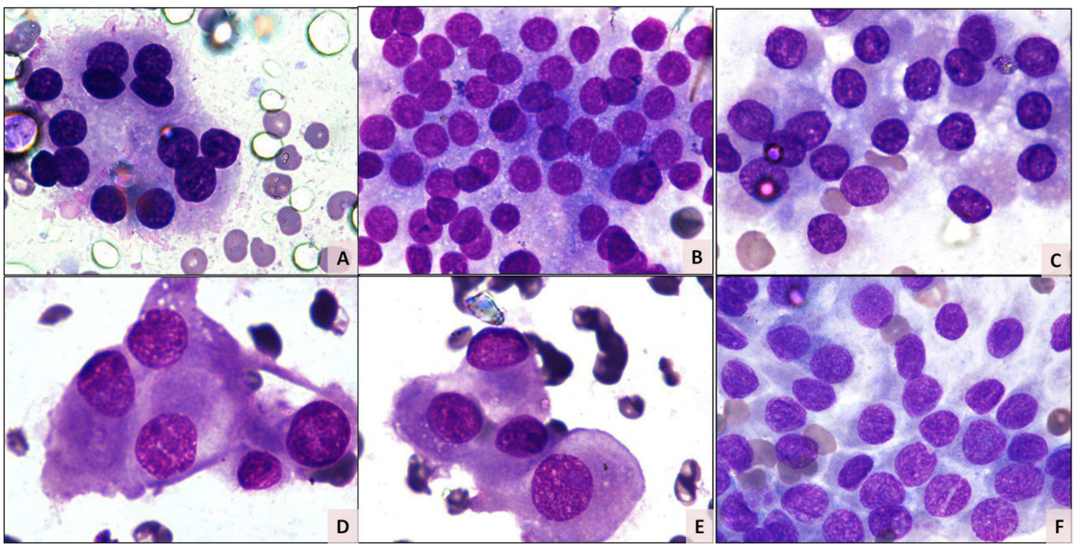

FIG. 1: Cytological photomicrographs, all Giemsa x 400. (A) Nodular goitre, (B and C) Follicular neoplasm, (D and E) Follicular carcinoma, (F) Papillary carcinoma

TABLE 1: Morphometric parameters in colloid goitre, follicular neoplasms and papillary carcinoma

Parameter	Colloid goitre	Follicular neoplasms	Papillary carcinoma
Nuclear morphometric parameters			
Perimeter	367.3 ± 361.2	336.47 ± 119.4	373.63 ± 77.95
Area	5722.87 ± 2000.2	5395.72 ± 1857.95	7749.69 ± 4052.21
Radius (centroid)	42.61 ± 6.43	41.52 ± 6.22	48.33 ± 9.4
Shape factor	0.66 ± 0.17	0.66 ± 0.17	0.68 ± 0.16
Roundness factor	1.37 ± 1.19	1.3 ± 0.43	1.24 ± 0.18
Compactness	0.89 ± 0.04	0.88 ± 0.04	0.87 ± 0.08
Circularity	0.12 ± 0.07	0.13 ± 0.07	0.19 ± 0.09
Elongation factor	1.15 ± 0.11	1.17 ± 0.11	1.26 ± 0.17
Textural parameters			
Red	105.72 ± 28.18	97.28 ± 28.3	129.83 ± 23.92
Green	45.62 ± 27.75	35.45 ± 24.69	72.66 ± 26.27
Blue	163.73 ± 19.72	153.4 ± 20.03	192.56 ± 13.94
Hue	303.33 ± 45.04	312.83 ± 53.46	309.58 ± 37.47
SRE	0.91 ± 0.02	0.91 ± 0.02	0.92 ± 0.01
LRE	1.52 ± 0.18	1.56 ± 0.16	1.43 ± 0.23
LGLRE	12.68 ± 7.63	12.98 ± 4.31	6.54 ± 2.36
HGLRE	12265.16 ± 5306.04	9803.01 ± 2628.39	17952.04 ± 3644.97
SRLGLE	11.26 ± 6.49	11.56 ± 3.64	5.98 ± 2.05
SRHGLE	11421.37 ± 5049.54	9054.51 ± 2512.81	16669.88 ± 3460.30
LRLGLE	21.62 ± 15.86	21.65 ± 9.31	9.61 ± 4.45
GLNU	109.83 ± 35.99	109.73 ± 32.28	134.80 ± 51.70
RLNU	4199.27 ± 1658.72	3828.93 ± 1424.62	5824.55 ± 2912.97

SRE= Short Run Emphasis; LRE= Long Run Emphasis; LGLRE= Low Gray Level Run Emphasis; HGLRE= High Gray Level Run Emphasis; SRLGLE= Short Run Low Gray Level Emphasis; SRHGLE= Short Run High Gray Level Emphasis; LRLGLE= Long Run Low Gray Level Emphasis; GLNU= Gray Level Non-Uniformity; RLNU= Run Length Non-Uniformity

neoplasms were small, no attempt was made to differentiate follicular adenoma and carcinoma.

As can be seen from Table 1, cases of papillary carcinoma showed the highest perimeter, area, radius and elongation factor while roundness factor was the lowest in papillary carcinoma among the three groups.

Discriminant analysis using both nuclear morphometric and textural parameters could classify 77.9% of cells correctly to a particular category. Among the individual groups, 66% cells of colloid goitre were correctly classified as goitre on discriminant analysis. The accuracy was better in the neoplastic category. Of the neoplastic cells, 84.5% of follicular neoplasms and 72.5% of cells of papillary carcinoma could be correctly classified to the respective category (Table 2).

The results of discriminant analysis are depicted in a tabular form in Table 2 and illustrated in Fig. 2, the latter showing minimal overlap in the colloid goitre and follicular neoplasm categories. The accuracy of cytomorphology was found to be 72% (36 of 50 cases), faring worst in follicular neoplasms.

DISCUSSION

Fine needle aspiration cytology (FNAC) is currently a reliable, reproducible, minimally invasive and hence cost-effective diagnostic modality in the assessment of nodular lesions of thyroid.[1] However, few authors have emphasized the limitations of this technique.[8] The usefulness of FNAC in thyroid has been limited by the difficulty in distinguishing follicular adenomas and multinodular goitre from well-differentiated follicular carcinoma and papillary carcinoma.[2] This difficulty stems from the lower incidence of anisokaryosis and poikilokaryosis in thyroid malignancies, both follicular and papillary and

TABLE 2: Results of discriminant analysis

My Exp Code	Predicted Group Membership 1	2	3	Total
% count				
1	**66.0**	20.2	13.8	100.0
2	12.0	**84.5**	3.4	100.0
3	3.8	23.8	**72.5**	100.0

1: nodular goitre; 2: follicular neoplasm; 3: papillary carcinoma

the many variations in cell size in benign thyroid lesions.[2]

Computer-aided morphometric analysis has been shown by some authors to improve the diagnostic yield of thyroid FNAC.[3,4,6,9] However, other authors have failed to confirm the utility of image analysis in the interpretation of thyroid FNAC.[5]

An early study by Wright *et al* employing morphometric analysis on both Papanicolaou and Giemsa-stained aspirates showed significant difference in mean nuclear area and perimeter between benign and malignant thyroid nodules. However, the wide variation in values was thought to limit morphometric assessment alone in prediction of malignancy.[4] Other authors have evaluated morphometry in follicular lesions. Coefficient of variation of nuclear area (NACV) was found to be suitable for differentiation of follicular carcinomas and adenomas at a cut-off point of 21.5%.[10] Karslioglu *et al* evaluated features like nuclear area, circular form factor, minimum and maximum feret and other such parameters in the differentiation of thyroid lesions in aspiration cytology. These authors utilized discriminant analysis and found that 75.6% of cases of papillary carcinoma were correctly classified, which improved to 84.8% by using a selected dataset. The correct classification rate of nodular goitre was 58.8% which improved to 68.7% with selected dataset. The authors concluded that morphometric studies can be

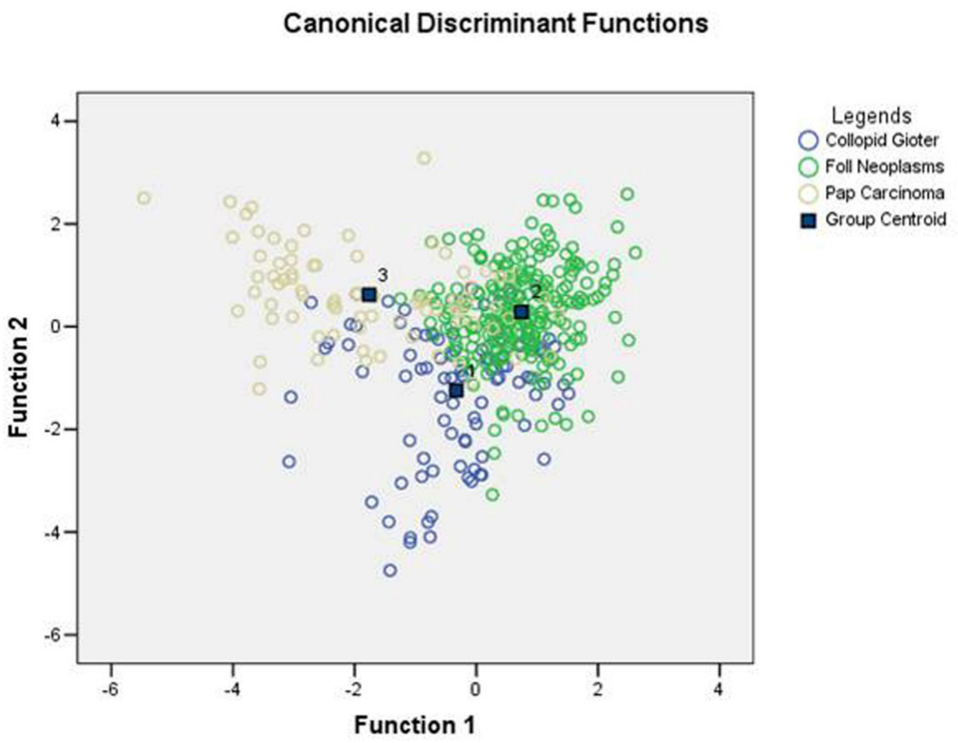

FIG. 2: Scatter plot to classify thyroid cases by first two functions on discriminant analysis taking all parameters together

planned with selection bias for cells showing maximum deviation from normal (or mean).[6]

The majority of the available morphometric studies have evaluated nuclear measurements including size, shape and other factors. Textural parameters, indicating chromatin distribution patterns have been utilized very rarely. A recent study by Murata *et al* used morphological abstraction method by factor analysis and combined morphometric and textural parameters. These authors showed that the nuclei of papillary carcinoma were larger, more irregular with higher contrast of chromatin pattern than those in the benign group. Follicular carcinoma, on the other hand, had larger nuclei and more monotonous chromatin pattern than the benign group.[7]

The present study, to the best of our knowledge, is the first attempt to combine morphometric and textural parameters with discriminant analysis for differential diagnosis of thyroid aspirates. In our study, 84.5% cells of follicular neoplasms and 72.5% of cells of papillary carcinoma were correctly classified. This is higher than the accuracy of cytomorphology alone (72% overall). Nuclear morphometric data suggests that nuclei of follicular carcinoma cells were more monomorphic, smaller with finer chromatin distribution than those of colloid goitre. On the other hand, papillary carcinoma nuclei were larger, less round with more heterogeneous chromatin distribution pattern. The results of the present study indicate that morphometry, including textural analysis can be a useful tool for cytologic diagnosis of thyroid lesions. Though this technique may not be applicable to diagnosis of a given case at present, further refinement of methods can achieve higher accuracy.

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