

Study of Fine Needle Aspiration Cytology of Thyroid Lesions by Morphometry

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Abstract: Fine Needle Aspiration Cytology (FNAC) is used as the screening procedure for thyroid nodules. FNAC utilizes size of cell and nuclei and shape of cells as a criterion for diagnosis and helps in the diagnosis of malignant lesions. Computer-assisted image morphometry is a tool for measuring cytological variables which characterise the size and shape of nuclei. This could be used along with FNAC for diagnosis of thyroid lesions. The main objectives of this study were: (a) to evaluate nuclear morphometric features i.e. major axis (MAJX), minor axis (MINX), nuclear area (NA), nuclear perimeter (NP), nuclear aspect ratio (ASPR) and nuclear roundness (NR) in FNAC of thyroid lesions and (b) to compare these parameters in various groups and to assess their diagnostic utility in thyroid lesions. We evaluated nuclear morphometric parameters using digital image analyzing software in 88 thyroid lesions in FNAC smears. There were: a) 15 cases of papillary Carcinoma, b) 15 cases of lymphocytic thyroiditis, c) 41 cases of colloid goiter and d) 17 cases of follicular lesions. Statistical evaluation using the analysis of variance (ANOVA) with turkey HSD test was carried out. ANOVA test showed that size related nuclear parameters: Mean MAJX, MINX, NA and NP with their variability were significantly different for the four groups. These parameters were significantly greater ($p < 0.05$) in cases of papillary carcinoma than other groups unlike shape related parameters, ASPR and NR. Size related parameters in lymphocytic thyroiditis was significantly greater than colloid goiter. Morphometric parameters can be gainfully exploited in the diagnosis of thyroid lesions.

Keywords: Nuclear morphometry, Fine needle aspiration cytology, Colloid Goiter, Follicular lesion thyroid, papillary carcinoma thyroid.

INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) is used as the initial screening procedure for palpable thyroid nodules. Incidentally detected thyroid nodules are very common, as much as 21% of the population and on ultrasonography this figure is much higher. All the nodules cannot be surgically operated as the frequency of thyroid nodules turning out malignant is only about 5% [1].

FNAC helps in diagnosis of malignant lesions and also helps in avoiding unnecessary surgery and morbidity associated with it. In India, the prevalence of a palpable thyroid nodule is approximately 12.2%, according to a study [2].

Image analysis is a method where images are obtained and objective and reproducible diagnostically important information is collected and analysed. Computer-assisted image morphometry is a tool for measuring cytological variables which characterise the size and shape of nuclei. As various cytological

techniques use size of cell and nuclei and shape of cells as a criterion for diagnosis, image morphometric analysis could be used as a supplement to FNAC for diagnosis of thyroid lesions [3]. FNAC technique, skill of the operator and preparation of specimen affect the diagnosis by FNAC [4].

In the present study, we evaluated the nuclear morphometric parameters in thyroid lesions to distinguish between benign and malignant lesions. The main objectives of the study were: (i) To evaluate: a) Major Axis (MAJX), b) Minor Axis (MINX), c) Nuclear Area (NA), d) Nuclear Perimeter (NP), e) Nuclear Aspect Ratio (NAR) and f) Nuclear Roundness (NR) using morphometric techniques. (ii) To compare these nuclear parameters statistically and their variability in various thyroid lesions.

MATERIALS AND METHODS

This was a cross-sectional study. All cases of thyroid nodules reporting to the selected hospital during one-year period of time were included in the study.

Ethical clearance was taken from the institutional ethical committee. Informed consent was taken.

FNAC was done either by palpation method or under ultrasound guidance. Those cases with inadequate material were excluded from the study. All smears were stained by Leishman Giemsa and Papanicolaou stain, and evaluated by pathologist to characterize the lesions into one of the four groups. These groups were colloid goitre, lymphocytic thyroiditis, follicular lesions and papillary carcinoma. a) 15 cases of papillary Carcinoma, b) 15 cases of lymphocytic thyroiditis, c) 41 cases of colloid goitre and d) 17 cases of follicular lesions. Nuclear morphometric parameters were evaluated using digital image analysing software in 88 thyroid lesions in smears stained by Leishman Giemsa [5].

Nuclear morphometric analysis

Nuclear morphometric analysis was performed without any information of cytological diagnosis. We took digital images of five high power field (400X) for each sample. (Figure.1A & 1B). Nonoverlapping nuclei were taken for finding the nuclear parameters. With the help of the cursor the nuclei in the recorded image were rounded and parameters were measured with the help of software. (Figure1C). A total of 100 nuclei were counted in each sample. This data was transferred to MS Excel sheet and analysed. Nuclei were analysed for MAJX, MINX, NP, NA, NR and NAR. The NR was defined as $[(4 \cdot \text{nuclear area}) / (\text{nuclear perimeter})^2] \times 100$ in percentage. Maximum NR is for a round nucleus and is 100. NAR was expressed as the ratio of the long axis to the short axis of a nucleus; Before measurement the system was calibrated for each resolution and digital magnification. MAJX, MINX, NA, NP, NAR and NR for each nucleus of every sample were analysed; We calculated the mean values of each sample with

standard deviation (SD). SD of the nuclear parameters: SD-MAJX, SD-MINX, SD-NA, SD-NP, SD-NAR and SD-NR was the variation of that parameter for that sample. To determine the differences in each group, statistical evaluation using the analysis of variance (ANOVA) with Turkey HSD test was carried out. The result was considered significant when $p < 0.05$.

RESULTS

The mean values of age and the studied parameters, MAJX, MINX, NA, NP, NAR, NR, SD-MAJX, SD-MINX, SD-NA, SD-NP, SD-NAR and SD-NR with their SD and range for the four groups, (a) Papillary Carcinoma, (b) Lymphocytic thyroiditis, (c) Colloid goiter and (d) Follicular lesions are presented in the Table2.

P values obtained by ANOVA test for all 4 studied groups are also showed in this table. P values of multiple comparisons for the parameters between the pairs of groups using post hoc Turkey HSD test are shown in the Table1. Figure2 shows the mean value and SD (error bar) of (a) MAJX, (b) NA, (c) NAR and (f) NR for the four groups.

ANOVA test showed the significant difference ($p < 0.05$) in the four groups for the size related nuclear parameters MAJX, MINX, NA, NP and their SDs; but no significant difference were observed for the shape related nuclear parameters NAR, NR and their SDs.

Mean size of nuclei for the Papillary Carcinoma was significantly larger than other three groups (i.e. Lymphocytic thyroiditis, Colloid goiter and Follicular lesions). But no difference was observed for NAR and NR. Mean nuclear size in Lymphocytic thyroiditis was significantly larger than Colloid goitre.

Table-1: p values of multiple comparisons for the parameters between the pairs of groups using post hoc Turkey HSD test

Sl No	Parameters (unit)	p(A vs B)	p (A vs C)	p (A vs D)	p (B vs C)	p(B vs D)	p (C vs D)
1	MAJX (micron)	0.0042*	<0.0001*	<0.0001*	0.0342*	0.1549	0.9912
2	MINX (micron)	0.0279*	<0.0001*	<0.0001*	0.0223*	0.2151	0.9091
3	NA (micron ²)	0.0030*	<0.0001*	<0.0001*	0.0208*	0.2667	0.8341
4	NP (micron)	0.0031*	<0.0001*	<0.0001*	0.0404*	0.1651	0.9934
5	NAR	0.9030	0.9684	0.9849	0.5765	0.9849	0.8001
6	NR (%)	0.4164	0.7863	0.2762	0.7871	0.9963	0.6008
7	SD-MAJX (micron)	0.7599	0.0183*	0.3306	0.2755	0.9019	0.7199
8	SD-MINX (micron)	0.4043	0.0039*	0.0391	0.3700	0.6870	0.9861
9	SD-NA (micron ²)	0.2942	0.0001*	0.0347*	0.1005	0.7844	0.5781
10	SD-NP (micron)	0.5028	0.0057	0.1991	0.3304	0.9465	0.6884
11	SD-NAR	0.9786	0.7939	0.9934	0.5020	0.9063	0.9197
12	SD-NR (%)	0.9976	0.9663	0.9425	0.9953	0.9828	0.9969

p (X vs Y), p value between X and Y groups. A- Papillary Carcinoma, B- Lymphocytic thyroiditis, C- Colloid goiter, D- Follicular lesions, * - difference is significant

Figure 1(A-C): Photo micrograph of malignant papillary carcinoma (A) and benign colloid goiter (B) FNAC sample (400 X magnification). Measurement of nuclear parameters (C) using Biowizard 4.1 software

Figure 2(A-D): Mean value and SD (error bar) of (a) MAJX, (b) NA, (c) NAR and (f) NR for the four groups. p values (post hoc Turkey HSD test) for the parameters between the pairs of groups are indicated in the figures

Table-2: The mean values of age and the studied nuclear parameters and variation of the nuclear parameters with SD and range for the four groups: (A) Papillary Carcinoma, (B) Lymphocytic thyroiditis, (C) Colloid goiter and (D) Follicular lesions. P values obtained by ANOVA test are also showed for all 4 studied groups. The parameters are expressed as: mean ± SD (minimum – maximum)

Sl No	Parameter (unit)	(A) Papillary Carcinoma n=15 Male=1, Female=14	(B) Lymphocytic thyroiditis n=15 Male=1, Female=14	(C) Colloid goiter n=41 Male=3, Female=38	(D) Follicular lesions n=17 Male=1, Female=16	p value (ANOVA Test)
1	Age (year)	41.1±11.2 (26-62)	36.5±14.0 (14-65)	43.5±12.1 (21-75)	47.9±14.3 (22-68)	0.1149
2	MAJX (micron)	12.03±0.73 (10.95-13.36)	10.66±0.91 (8.95-11.75)	9.76±1.01 (7.79-11.82)	9.85±1.51 (7.31-12.30)	<0.0001*
3	MINX (micron)	9.40±0.60 (8.79 - 10.72)	8.43±0.74 (7.05-9.47)	7.60±0.88 (5.58-9.26)	7.78±1.37 (5.33-10.00)	<0.0001*
4	NA (micron ²)	84.64±10.37 (70.95-105.66)	67.22±11.16 (46.27-81.36)	55.40±11.70 (32.36-78.24)	58.62±19.49 (28.65-94.40)	<0.0001*
5	NP (micron)	37.54±2.39 (34.05-41.98)	32.98±3.00 (27.27-36.79)	30.14±3.31 (23.76-37.07)	30.40±4.84 (22.33-38.21)	<0.0001*
6	NAR	1.30±0.04 (1.21-1.35)	1.28±0.06 (1.19-1.44)	1.30±0.06 (1.20-1.47)	1.29±0.09 (1.18-1.51)	0.5804
7	NR (%)	74.67±2.67 (71.32-79.83)	76.68±3.13 (69.34-82.12)	75.67±3.66 (67.24-82.51)	76.95±4.30 (65.75-83.19)	0.2535
8	SD-MAJX (micron)	1.85±0.56 (1.13-3.54)	1.66±0.40 (0.93-2.28)	1.36±0.39 (0.68-2.51)	1.53±0.86 (0.73-4.46)	0.0224*
9	SD-MINX (micron)	1.59±0.42 (1.17-2.87)	1.38±0.24 (0.84-1.80)	1.20±0.31 (0.71-2.01)	1.23±0.52 (0.72-2.67)	0.0054*
10	SD-NA (micron ²)	26.74±9.63 (15.39-54.93)	21.05±5.82 (8.97-30.80)	14.88±5.21 (6.65-27.32)	18.13±15.08 (7.60-69.01)	0.0003*
11	SD-NP (micron)	6.34±1.69 (4.28-11.51)	5.45±1.28 (3.07-7.42)	4.57±1.26 (2.22-8.36)	5.12±2.78 (2.46-14.49)	0.0095*
12	SD-NAR	0.18±0.03 (0.13-0.24)	0.17±0.05 (0.13-0.31)	0.19±0.05 (0.12-0.33)	0.18±0.05 (0.12-0.27)	0.5155
13	SD-NR (%)	10.18±1.11 (8.43-11.89)	10.27±1.12 (8.81-13.17)	10.36±1.34 (8.23-13.09)	10.44±1.34 (8.26-12.55)	0.9443

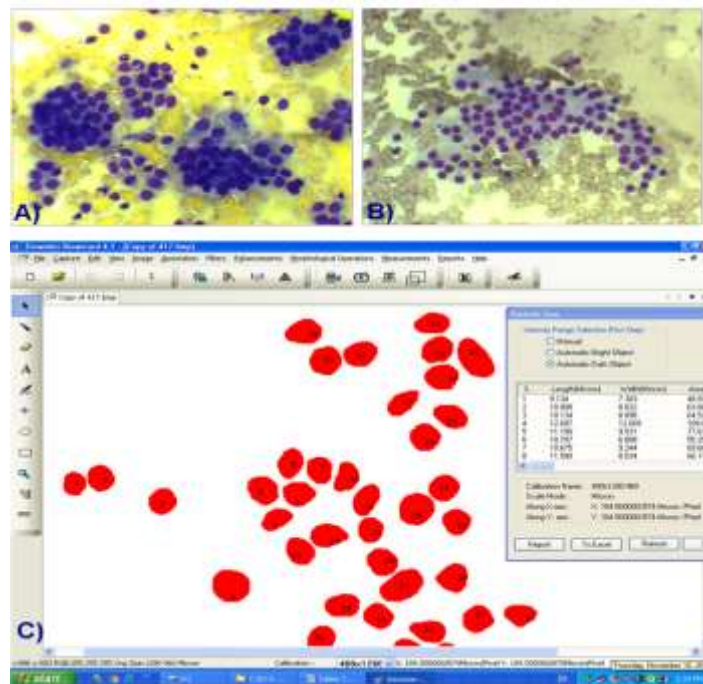


Fig-1(A-C): Photo micrograph of malignant papillary carcinoma (A) and benign colloid goiter (B) FNAC sample (400 X magnification). Measurement of nuclear parameters (C) using Biowizard 4.1 software

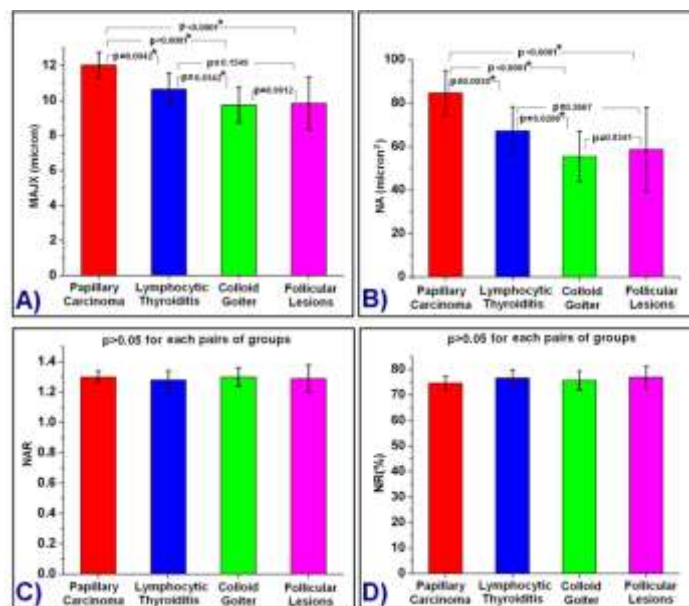


Fig-2(A-D): Mean value and SD (error bar) of (a) MAJX, (b) NA, (c) NAR and (d) NR for the four groups. p values (post hoc Turkey HSD test) for the parameters between the pairs of groups are indicated in the figures

DISCUSSION

Thyroid nodules are very common with some presenting as palpable nodules while others are discovered only on ultrasound or some other radio imaging methods. 7-15% of these thyroid nodules are detected to have cancer of which 90% are papillary and follicular carcinomas [6].

FNAC is useful in thyroid nodules as it helps avoid unnecessary surgery. Despite its usefulness the rate of false negative tests is high as assessment is subjective. Computer-assisted image morphometry can

measure many variables like the size and shape of nuclei and these have been found useful in differentiating benign from malignant cells. It adds objectivity to the morphological assessment. In some previous quantitative studies in thyroid, researchers have investigated the role of morphometry in diagnosis of follicular lesions and found statistically significant differences between adenomatous nodule and follicular adenoma, follicular adenoma and follicular variant of papillary carcinoma and it has been suggested that morphometry could play a role in preoperative assessment of these lesions [5]. Though computer-

assisted image analysis has been found to be a useful supplement to diagnosis of thyroid lesions, very few morphometric studies in thyroid are found in world literature [6].

Priya *et al.* have estimated cyto-morphometric features in FNAC of thyroid and found that quantitative estimation of nuclear features may help in assessment before surgery and thus may add to the diagnostic accuracy [3].

Khatri *et al.* found significant differences in nuclear parameters between benign and malignant lesions of thyroid [7]. We found significantly larger nuclear size in malignant lesions than the benign lesions. Though, another study by Kefeli *et al.* [8] indicated that the ratio of MaxD/MinD (i.e. aspect ratio) was a distinct nuclear morphometric feature for distinguishing papillary carcinoma from other thyroid lesions, but we did not find so in our study. Hence the nuclear size related parameters can be utilized for differentiation of malignancy in borderline cases using FNAC materials.

After searching our records, we found 18 biopsy reports of the total 88 studied FNAC cases; where 9 were reported Papillary Carcinoma, 2 were reported Lymphocytic thyroiditis, 3 were Colloid goiter and 4 were Follicular lesions in FNAC diagnosis. We found disagreement in two cases out of the 18. First case was reported as Papillary Carcinoma in FNAC diagnosis and was diagnosed as Hashimoto's thyroiditis on histopathological diagnosis. For this particular case: MAJX=11.13 μm , MINX =8.79 μm , NA=71.82 μm^2 and NP=34.73 μm ; and these parameters were much smaller than the mean values of the respective size related parameters of the Papillary Carcinoma. Second case was reported as Colloid Goitre in FNAC diagnosis and found as Papillary Carcinoma in histological diagnosis. For this particular case: MAJX=10.83 μm , MINX =8.43 μm , NA=70.04 μm^2 and NP=34.08 μm ; and these parameters were much larger than the mean values of the respective size related parameters of the Colloidal Goiter. Hence morphometry of such suspicious cases in FNAC could provide a better direction for a pathologist for accurate diagnosis.

The SD of the size related parameters is a quantitative measure of nuclear pleomorphism, which was significantly higher in malignant cases [9]. As all cases whose FNAC was done were not operated at this centre, therefore, histopathological correlation for all samples could not be done.

There is a possibility to develop an automatic system to evaluate mean nuclear size from the FNAC slide, which could be incorporated as a new way for the screening for malignancy in thyroid.

CONCLUSIONS

Morphometry is an important complement to fine needle aspiration cytology of thyroid lesions as size related parameters and their variability are found to be significantly larger in malignant cases. These parameters can be gainfully exploited and objective understanding and in the diagnosis of various thyroid nodules.

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