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Original Article

Pathology Section

Nuclear Morphometry as an Adjunct to Cytomorphology in the Diagnosis of Thyroid Lesions

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ABSTRACT

Introduction: Fine Needle Aspiration Cytology (FNAC) is a reliable and reproducible diagnostic technique for thyroid lesions. Recently, it has been suggested that evaluation of nuclear features may enhance the diagnostic utility of FNAC. However, the evaluation of nuclear morphometry is not well established in thyroid cytology.

Aim: To evaluate the role of nuclear morphometry in cytological evaluation of thyroid lesions.

Materials and Methods: This was a cross-sectional study conducted over a period from March 2019-February 2020 at Hamdard Institute of Medical Sciences and Research, New Delhi, India. Morphometry was done on 40 cases of thyroid aspirates which had histopathological concordance. Computerised nuclear morphometry was done by using photographs captured under Motic photomicrography system. Six parameters were measured- nuclear area, nuclear perimeter, minimal nuclear diameter, maximal nuclear diameter, nuclear compactness and LS ratio (Largest to Smallest dimension ratio). Data were entered in spreadsheet and then analysed using Statistical Package for Social Sciences (SPSS) version 20.0.

Results: Out of total 40 thyroid aspirates studied, included non neoplastic (19 cases), benign (12 cases) and malignant lesions (9 cases). All nuclear morphometry parameters comprising of nuclear area, nuclear perimeter, minimal nuclear diameter, maximal nuclear diameter showed an increasing trend from non neoplastic to benign to malignant with a statistically significant difference between benign and malignant groups (p-values <0.05) except for LS ratio and nuclear compactness.

Conclusion: Nuclear morphometry can aid in cytological diagnosis of thyroid lesions. If used judiciously, quantitative estimation of cytological nuclear features can be helpful in assessing thyroid lesions preoperatively thus complementing its cytomorphological features.

Keywords: Aspiration cytology, Histopathological, Photomicrography, Thyroid aspirates

INTRODUCTION

Thyroid disease is a frequently encountered clinical problem in general population and around 42 million people suffer from it in India [1]. The prevalence of palpable thyroid nodule in India is approximately 12.2%. However, the incidence of thyroid cancer is only 8.7 cases per 100,000 population per year [2]. Thyroid nodules are common findings in clinical practice and these nodules can represent both benign and malignant pathologies. Further evaluation is required with a comprehensive ultrasound examination and FNAC, with the latter remaining a well established Outpatient Department (OPD) procedure used in the diagnosis of palpable thyroid lesions. It's greatest merit is in identifying a substantial proportion of thyroid nodules as benign thus reducing unnecessary thyroid surgeries [3].

Computerised nuclear morphometry is a cost effective, objective, and reproducible tool for the evaluation of nuclear features which can enhance the interpretation of morphological features by transformation of pathological changes in cells to a quantitative form. Nuclear morphometry is the measurement of nuclear parameters by image analysis. Many nuclear parameters (e.g., shape, size) can be analysed using nuclear morphometry [4]. 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 [5]. The validity and use of nuclear morphometry has already been established in cancers of breast and skin, however its role in assessing thyroid lesions is in the naive stage. The evaluation of nuclear morphometry is not well established in thyroid cytology. Recently, it has also been suggested that nuclear morphometric parameters may allow differentiation between various thyroid lesions.

Nuclear morphometry when done on thyroid aspirates can evaluate the significance of nuclear parameters improving the prediction of thyroid malignancy. Application of morphometry to cytology as an adjunct to morphological criteria may be useful in the accurate diagnosis of thyroid tumours. Very few studies have been done to evaluate nuclear features in thyroid aspirates using morphometry in order to differentiate non neoplastic, benign and malignant lesions [4,5]. Thus, we planned to evaluate the role of nuclear morphometry in thyroid FNACs which were classified according to the recent 2017 Bethesda system for reporting thyroid cytopathology and assessing nuclear features among benign and premalignant through malignant groups [6]. The aim of the study was to evaluate the significance of nuclear morphometric parameters in cytological aspirates of thyroid.

MATERIALS AND METHODS

The present cross-sectional study was conducted from March 2019 to February 2020, in the Department of Pathology on patients with thyroid swellings attending outpatient and inpatient services of the Department of Surgery and Otorhinolaryngology and Head and Neck Surgery, Hamdard Institute of Medical Sciences and Research and associated Hakeem Abdul Hameed Centenary Hospital, New Delhi. The study commenced after taking approval of Ethics and Scientific Committee of the Institute. (JH-IEC No 14/18).

Inclusion criteria: All those thyroid cytology cases (Bethesda category II to VI) in whom histopathology was also done during the present study period of one year were included in the study.

Exclusion criteria: Patients who did not undergo histopathological evaluation were excluded from the study.

Sample size calculation: The study participants were chosen by using convenience sampling technique.

Study Procedure

The present study characterised the cytological features of thyroid lesions ranging from benign through premalignant to malignant.

The lesions were evaluated and classified according to the recent Bethesda System for Reporting Thyroid Cytopathology [6]. An objective method of morphometry was also done in all those cases of FNAC thyroid where histopathology was available.

Thus, a total of 40 cases of thyroid FNACs with histopathological concordance were studied. Based on histopathology, they were classified into non neoplastic, benign and malignant.

Cytology slides stained with Giemsa, Haematoxylin and Eosin (H&E) were selected. Computerised nuclear morphometry was done by using photos captured under Motic microscope. An average of 10 microscopic fields; at magnification ×400 were captured for each case. At least 50 nuclei were analysed per case. Cells which were not overlapping with intact whole nuclei were considered. These cells were outlined using the Sketch command by the computer mouse in the Motic images plus 3.0 and following parameters were measured-nuclear area, nuclear perimeter, minimum nuclear diameter and maximum nuclear diameter. Four parameters i.e., nuclear area, nuclear perimeter, minimal nuclear diameter and maximal nuclear diameter were measured from the software and were saved in the excel sheet and were later used to calculate the other two parameters: LS ratio and nuclear compactness. LS ratio=maximal nuclear diameter/minimal nuclear diameter. Nuclear compactness was calculated by the formula (mean nuclear perimeter2/mean nuclear area). Measurements were calibrated in terms of micrometer. However, person doing nuclear morphometry was not blinded to the histopathology diagnosis.

STATISTICAL ANALYSIS

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS inc., Chicago Illinois, USA). Continuous variables were expressed as Mean±SD and categorical variables were summarised in percentages. Analysis of variance (ANOVA) was employed to find the significance of study parameters between three groups. Post-hoc Tukey's test was used to find the pairwise significance. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In the present study, nuclear morphometry was done in 40 thyroid aspirates in whom follow-up histopathological examination was available. They were divided into three groups based on histopathology- non neoplastic (19 cases), benign (12 cases) and malignant (nine cases).

Although there was mild increase in all morphometric parameters on progression from non neoplastic to benign category however it was not found to be statistically significant, (p-value >0.05). Thus, showing there is some overlap between non neoplastic and benign categories and it may be difficult to differentiate between these categories based on nuclear morphometric parameters [Table/Fig-1].

Parameter	Non neoplastic		Benign		p-value
	Mean	SD	Mean	SD	
Nuclear area	54.23	15.68	62.62	26.78	0.279
Nuclear perimeter	32.15	4.88	33.79	7.337	0.459
Minimal nuclear diameter	4.93	0.809	5.20	0.989	0.405
Maximal nuclear diameter	8.29	1.186	9.46	2.061	0.053
LS ratio	1.90	0.489	1.96	0.327	0.721
Nuclear compactness	19.82	1.23	19.95	1.228	0.769

[Table/Fig-1]: Comparison of morphometric parameters between non neoplastic and benign groups.
LS Ratio: Largest to smallest dimension ratio; SD: Standard deviation; Post-hoc Tukey's test used

In the present study on non neoplastic vs malignant group, it is observed that the nuclear area, nuclear perimeter, minimal nuclear diameter, maximal nuclear diameter were significantly different (p-value <0.05) with the malignant group showing higher values. However, nuclear LS ratio and nuclear compactness did not show a statistical significance when compared between the groups. This is shown in [Table/Fig-2].

Parameter	Non neoplastic		Malignant		p-value
	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	
Nuclear area	54.23	15.68	99.83	49.77	0.001*
Nuclear perimeter	32.15	4.88	43.60	11.54	0.001*
Minimal nuclear diameter	4.93	0.809	5.91	1.634	0.043*
Maximal nuclear diameter	8.29	1.186	12.63	4.042	<0.001*
LS ratio	1.90	0.489	2.25	0.784	0.164
Nuclear compactness	19.82	1.23	20.48	1.905	0.291

[Table/Fig-2]: Comparison of morphometric parameters between non neoplastic and malignant groups.
LS ratio: Largest to smallest dimension ratio; SD: Standard deviation
*Statistically significant difference (p-value <0.05); Post-hoc Tukey's test used

In the present study, it was observed that the nuclear area, nuclear perimeter, minimal nuclear diameter, maximal nuclear diameter when compared between benign and malignant groups were found to be statistically significant (p-value <0.05). However, nuclear LS and nuclear compactness did not show a statistical significance when compared between the groups. This is shown in [Table/Fig-3].

Parameter	Benign		Malignant		p-value
	Mean (µm)	SD (µm)	Mean (µm)	SD (µm)	
Nuclear area	62.62	26.78	99.83	49.77	0.043*
Nuclear perimeter	33.79	7.337	43.60	11.54	0.032*
Minimal nuclear diameter	5.20	0.989	6.41	1.234	0.038*
Maximal nuclear diameter	9.46	2.061	12.63	4.042	0.025*
LS ratio	1.96	0.327	2.25	0.784	0.254
Nuclear compactness	19.95	1.228	20.48	1.905	0.463

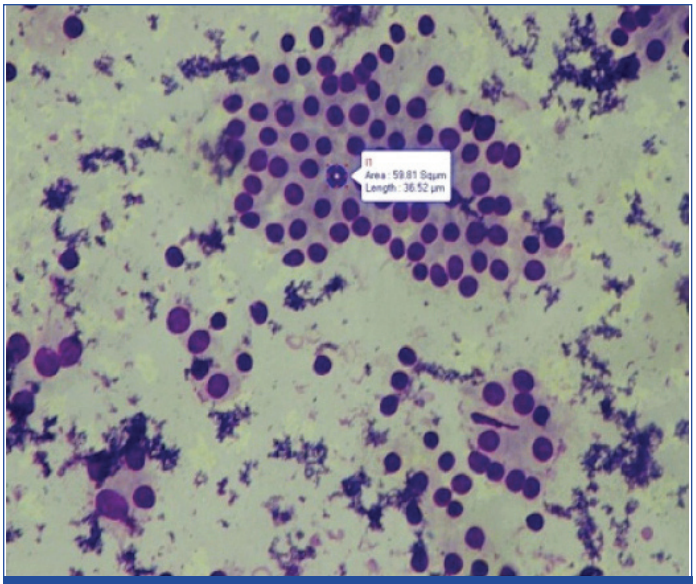
[Table/Fig-3]: Comparison of morphometric parameters between benign and malignant groups.
*Statistically significant difference (p-value <0.05); Post-hoc Tukey's test used

The mean nuclear area ranged from 54.23±15.68 µm in non neoplastic cases to 62.62± 26.78 µm in benign neoplastic cases and 99.83±49.77 µm in malignant cases [Table/Fig-4]. Mean nuclear perimeter ranged from 32.15±4.88 µm in non neoplastic cases to 33.79±7.33 µm in benign and 43.60±11.54 µm in malignant cases. [Table/Fig-4,5] shows nuclear morphometric parameters in a non neoplastic lesion i.e., colloid goitre. [Table/Fig-6,7] shows morphometric parameters in a benign neoplastic lesion i.e., follicular neoplasm and in a malignant neoplastic lesion i.e., papillary thyroid carcinoma. It was observed that nuclear area showed progressive increase across various categories ranging from non neoplastic to malignant.

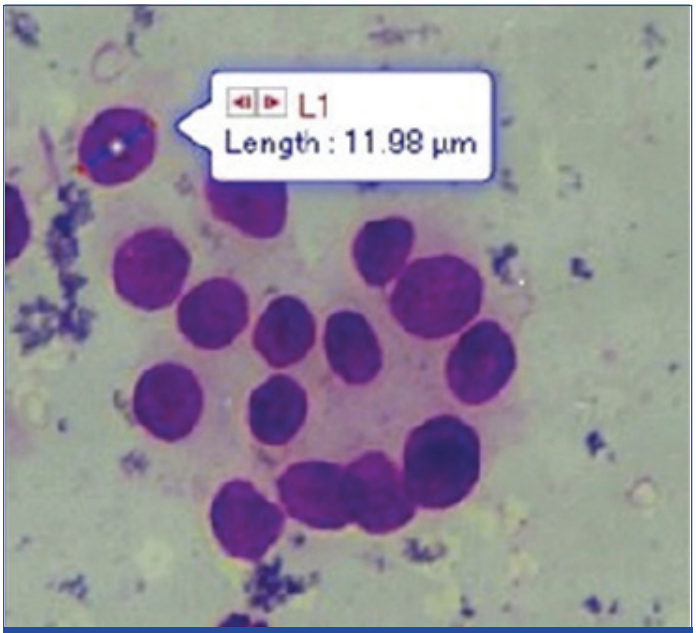
Parameter	Non neoplastic		Benign		Malignant	
	Mean (µm)	SD	Mean (µm)	SD	Mean (µm)	SD
Nuclear area	54.23	15.68	62.62	26.78	99.83	49.77
Nuclear perimeter	32.15	4.88	33.79	7.33	43.60	11.54
Minimal nuclear diameter	4.93	0.80	5.20	0.98	6.41	1.23
Maximal nuclear diameter	8.29	1.18	9.46	2.061	12.63	4.042
LS ratio	1.90	0.48	1.96	0.32	2.25	0.78
Nuclear compactness	19.82	1.23	19.95	1.22	20.48	1.90

[Table/Fig-4]: Nuclear morphometric parameters in thyroid aspirates with histopathological follow-up (n=40).
LS: Largest to smallest dimension ratio

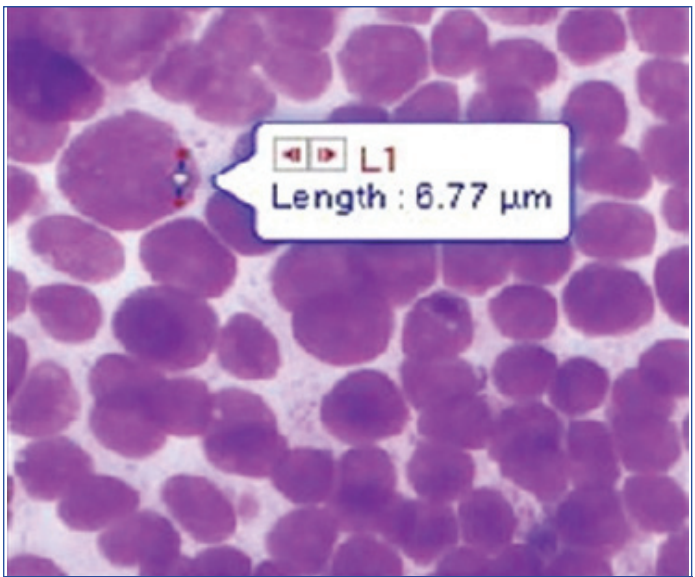
Mean minimal nuclear diameter ranged from 4.93±0.80µm in non neoplastic to 5.20±0.98 µm in benign and 6.41±1.23 µm in malignant cases [Table/Fig-4,7]. Mean maximal nuclear diameter ranged from 8.29±1.18 µm in non neoplastic to 9.46±2.06 µm in benign neoplastic



[Table/Fig-5]: Nuclear morphometry showing nuclear area and perimeter in a case of colloid goitre (Giemsa, x200).



[Table/Fig-6]: Nuclear morphometry showing nuclear area and perimeter diameter in a case of Follicular Neoplasm (Giemsa, x400).



[Table/Fig-7]: Nuclear morphometry showing minimum nuclear diameter in a case of papillary thyroid carcinoma (Giemsa, x400).

and 12.63±4.04 µm in malignant cases. The LS ratio ranges from 1.90±0.48 µm in non neoplastic to 1.96±0.32 µm in benign and 2.25±0.78 µm in malignant cases. Nuclear compactness ranges from 19.82±1.23 µm in non neoplastic to 19.95±1.22 µm in benign and 20.48±1.90 µm in malignant cases.

DISCUSSION

Nuclear morphometry by computerised image analysis is now increasingly being used to evaluate nuclear parameters. It may prove to be helpful in quantifying a number of parameters specially those related to nuclear size and shape [7]. Few of the previous studies showed that nuclear area, nuclear perimeter, minimal nuclear diameter and maximal nuclear diameter, were significantly different across benign and malignant thyroid pathologies, with malignant group having higher values [3].

The present study showed that among groups, mean nuclear area, mean nuclear perimeter, mean minimal nuclear diameter and maximal nuclear diameter were higher in malignant group compared to that of non neoplastic group which was similar to the study of Yashaswini R et al., [4]. Both benign and malignant thyroid lesions showed a higher Standard Deviation (SD) of mean minimal diameter and maximal diameter as compared to that of non neoplastic lesions. The SD of mean nuclear area among non neoplastic, benign, and malignant groups was 15.68 µm, 26.78 µm and 49.77 µm. Among calculated parameters, mean LS ratio and nuclear compactness did not show any statistical significance between groups, all other remaining parameters had good statistical correlation which was in concordance with study by Yashaswini R et al., [4].

The higher mean nuclear area in malignant group suggests nuclear size enlargement, which is a feature of malignant lesions. However, the role of nuclear morphometry is limited in distinguishing follicular adenoma vs follicular carcinoma and diagnosing cases of follicular variant of papillary carcinoma thyroid where the nuclear features of papillary carcinoma is not overt.

Fourteen cases of conventional papillary carcinoma and five cases of tall cell variant of papillary carcinoma were studied by Dina R et al., [8]. They concluded that nuclear area and nuclear diameter was higher in tall cell variant as compared to conventional cases of papillary carcinoma thus suggesting that malignant lesions had higher range of nuclear area and diameter [8]. However, in the present study we had 11 cases of conventional papillary thyroid carcinoma and there were no variants of papillary thyroid carcinoma. Rajesh L et al., found in his study that convex nuclear area and perimeter of follicular hyperplasia when compared with that of follicular neoplasm and follicular variant of papillary carcinoma were much lower [9].

For a better use and understanding of the nuclear morphometry, Karslioglu Y et al., suggested that morphologic examination is based primarily on selection. There maybe a dilution effect if morphometric data from all the cells is used and therefore, it will be more useful if morphometric studies are carried out with a proper selection "bias"[10].

In one of the studies, Priya SS and Sundaram S evaluated the cytomorphometric features in 36 FNAC of thyroid lesions. Findings revealed that when correctly applied, quantitative estimation of cytological nuclear features can play an important role in preoperative assessment and can complement morphological features in thyroid lesions [7].

In another study, Aiad HA et al., studied the role of nuclear morphometry in differential diagnosis of thyroid lesions having predominant follicular pattern. The parameters related to nuclear size (area, perimeter, maximal and minimal diameter, nuclear size) and shape (LS ratio, Form_AR) were significantly higher in neoplastic group follicular variant of papillary carcinomas (FVPC), Follicular Carcinomas (FC), Follicular Adenoma (FA) when compared to non neoplastic group (NG) p-value <0.05. The perimeter was the most reliable parameter. Within the neoplastic group, nuclear area and size were the most reliable parameters for differentiation between

FVPC and FA. However, there was no quantitative difference between FC and FA. Authors, thus, concluded that nuclear morphometric parameters may help in differentiation between neoplastic and non neoplastic thyroid lesions and between FVPC and follicular neoplasms (FC and FA) but they have no value in differentiation between FC and FA [11].

Deka L et al., conducted nuclear morphometry and texture analysis on cytological smears of 50 cases of thyroid neoplasms which revealed that papillary thyroid carcinoma had the highest perimeter, area, radius and elongation factor as compared to other thyroid lesions. Study also revealed that based on the nuclear morphometric and textural parameters 77.9% of cells could be correctly classified to their lesion category [12].

In the latest study by Yashaswini R et al., computerised nuclear morphometry was done on 81 cases which had confirmed cytohistological correlation, using Aperio computer software. Minimal nuclear diameter, maximal nuclear diameter, nuclear perimeter, and nuclear area were significantly higher in malignant group compared to non neoplastic and benign group which was seen in the present study too [4].

However, in a recent study conducted by Razavi MA et al., they concluded that nuclear morphometry is not of reliable diagnostic value in distinguishing malignant and non malignant thyroid nodules which was discordant with this study [13]. Hend S et al., deduced that morphometric measurements can aid in the differentiation between benign and malignant thyroid lesions especially follicular patterned lesions [14]. Bhatia JK et al., suggested that an automatic system to evaluate mean nuclear size from the FNAC slide can be incorporated as a new way for screening of malignancy in thyroid [15].

In the present study, it was found that false negative cases on cytology had nuclear morphometric parameters similar to malignant group. Thus, it may be deduced from the present study, that in cases wherever there is diagnostic dilemma regarding the Bethesda category of thyroid lesions especially in cytologically indeterminate cases, nuclear morphometry may be used as an adjunct along with cytomorphology for improved diagnostic accuracy.

Limitation(s)

It was a single centre hospital based study where sample size was small as histopathological follow-up was available in only limited number of cases.

CONCLUSION(S)

To conclude, this study suggests that nuclear morphometric parameters show significant difference across benign and malignant thyroid pathologies. Morphometry is helpful in cases which are of diagnostic challenge on cytology due to the overlapping cytological features and can play an important role in preoperative assessment of thyroid lesions. When correctly applied with appropriate cut-offs, morphometry can act as an adjunct to morphological features in thyroid lesions and minimise the number of diagnostic thyroid surgeries.

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