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Original Research Paper

Pathology

CYTOLOGICAL STUDY OF THYROID LESIONS AS CLASSIFIED BY THE BETHESDA SYSTEM WITH HISTOLOGICAL CORRELATION AND MORPHOMETRY

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ABSTRACT The thyroid gland is located in the anterior neck, composed of left and right lobes and a small isthmus, weighing approximately 12-20gm in adults. It is composed of follicles ranging from uniform to variable in size and are lined by cuboidal to low columnar epithelium, which is filled with thyroglobulin. Fine needle aspiration cytology (FNAC) of the thyroid is the single best test to aspirate samples for cytological assessment but has been a subject of much debate for last forty years. Now, the Bethesda system has been developed for thyroid FNA diagnosis. FNAC has its limitations in differentiating thyroid follicular lesions between adenoma and carcinoma and in predicting various facets of thyroid papillary lesions. The possibility of morphometric analysis of FNAC smears in overcoming these limitations as a valuable preoperative diagnostic tool was investigated. The Mean Nuclear Area (MNA) was calculated from 100 nuclei in each case. FNA of the thyroid remains the safest and most cost-efficient manner in which to stratify thyroid nodules for surgical excision. The Bethesda system has standardized the reporting nomenclature for thyroid FNAC that corresponds with specific cytomorphologic criteria and risk of malignancy. Morphometric analysis in case of thyroid lesions is of definite benefit to the patient with regard to the future management as it aids an early pre-operative diagnosis with a high rate of accuracy.

KEYWORDS : Fine needle aspiration cytology (FNAC), Thyroid gland, Morphometry, The Bethesda System.

INTRODUCTION:

The thyroid gland is located in the anterior neck, composed of left and right lobes and a small isthmus, weighing approximately 12-20gm in adults. It is composed of follicles ranging from uniform to variable in size and are lined by cuboidal to low columnar epithelium, which is filled with thyroglobulin. Thyroid nodules are common, seen in about 8.5% of the population.[1] Whenever a patient presents with a thyroid swelling, the task of the clinician is to distinguish a benign nodule from the malignant one. FNAC of the thyroid has gained wide acceptance due to its rapidity and cost-effectiveness in distinguishing neoplastic from non-neoplastic nodules and identifying those patients requiring surgery. FNAC, by giving direct morphological information has supplanted most other tests for preoperative evaluation of thyroid nodules. The reporting of thyroid cytology has been subject of much debate for last forty years. Now there is the Bethesda system of thyroid FNAC diagnosis. FNAC smear is considered satisfactory if 6 or more groups of 10 follicular cells each are present on a minimum of two slides.[2] In FNAC, both false positive and negative results occur.[3] FNAC has its limitations in differentiating thyroid follicular lesions between adenoma and carcinoma and in predicting various facets of thyroid papillary lesions. As observed by Klencka D S et al [4], karyometric analysis in the cytologic diagnosis of thyroid lesions increase the probability of a correct diagnosis with FNAC examination. The possibility of morphometric analysis of FNA cytologic smears in overcoming these limitations as a valuable preoperative diagnostic tool for various thyroid lesions has aroused our interest. The application of morphometry in tumor pathology includes its use in studying the biology of tumors, in creating tumor classifications, in creating methods for the identification of a tumor in the diagnostic context, and in characterizing diagnostic histopathology in absolute terms.[5]

MATERIAL & METHODS:

The study was conducted in the Dept of Pathology between January 2017 to June 2018.

Thyroid lesions preoperatively diagnosed by morphometry aided cytology as per categories of The Bethesda System for Reporting Thyroid Cytology and later undergoing surgery were selected.

The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories

I. Nondiagnostic or Unsatisfactory

-Cyst fluid only, Virtually acellular specimen, Other (obscuring blood, clotting artifact)

II. Benign

- Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)
- Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
- Consistent with granulomatous (subacute) thyroiditis /-Other

III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance

IV. Follicular Neoplasm or Suspicious for a Follicular Neoplasm

- Specify if Hürthle cell (oncocytic) type

V. Suspicious for Malignancy

- Suspicious for papillary carcinoma /-Suspicious for medullary carcinoma
- Suspicious for metastatic carcinoma /-Suspicious for lymphoma
- Other

VI. Malignant

- Papillary thyroid carcinoma /-Poorly differentiated carcinoma
- Medullary thyroid carcinoma /-Undifferentiated (anaplastic) carcinoma
- Squamous cell carcinoma /-Carcinoma with mixed features (specify)
- Metastatic carcinoma /-Non-Hodgkin lymphoma /-Other

Study variables:

Patient particulars: Age, Sex, Occupation, Weight, Socioeconomic status were noted.

Clinical examination included size of the lesions, fixity, duration, move with deglutition, presence of laterisation, metastasis (if any). Hematological and biochemical parameters and Ultrasonographic features were recorded.

FNAC with Cytopathology. Morphometric analysis with Mean Nuclear area was calculated. At least 100 intact nuclei in each slide were considered. Histopathological examinations. 100 cases were studied for association of various factors. Among them, morphometry were done in all cases.

Each FNAC case was stained with May-Grunwald-Giemsa (M.G.G) stain.

Result: Nuclear chromatin – dark blue; Cytoplasm – light blue. Histopathological evaluation of post operative thyroid specimens was done by Routine staining of all sections by Haematoxylin and Eosin (H & E) stain. Result: Nuclei - blue/black; Cytoplasm –varying shades of pink or clear due to presence of glycogen or fat. Muscle fibres - deep pink/red; RBC – orange / red; Collagen fibers – pink; Fibrin- deep pink.These slides were studied for Histopathological examination and were compared with those of FNAC findings. Morphometric analyses were done on these cytological smears with the aid of an ocular morphometer attached to the **10x** eye-piece of a microscope using a **40x** high-dry objective. The ocular morphometer was calibrated using a stage calibration slide provide with it. **One small division on morphometer equaled 2.45 micron**. 100 random nuclei from each case were subjected to analysis. Major and Minor axes, Mean Nuclear Diameter (MND) and Mean Nuclear Area (MNA) were observed in the various thyroid lesions. Any nucleus of a cell was assumed to be an ellipse. The Nuclear Area (A) was calculated by the formula : $\mathbf{A = \pi \times a \times b}$ where a and b are the semi largest and semi smallest nuclear diameters. All significant data were properly tabulated in master chart and represented by relevant tables and charts.

RESULT AND ANALYSIS:

The age of the patients ranged from 10 years to 82 years. Maximum patients were in the age range between 21-40years. 84% of cases were females and 16% were males.

Table 1 shows that 17 cases of Colloid goitre were correctly diagnosed by FNAC. Out of the other 15 cases of colloid goitre diagnosed by FNAC, 4 were Adenomatoid goitre (non-neoplastic), 3 were proved to be Follicular adenoma (benign neoplastic), 7 were diagnosed as follicular carcinoma and 1 were proved as papillary carcinoma (malignant neoplastic) on histology. 8 cases of Adenomatoid goitre were correctly diagnosed by FNAC. Out of the other 4 cases diagnosed by FNAC, 3 were proved as colloid goitres and 1 was diagnosed as follicular adenoma on histology. 5 cases of Hashimoto thyroiditis were correctly diagnosed by FNAC. Other 3 cases were diagnosed by FNAC, as Follicular carcinoma on histology. 2 cases of thyroglossal cyst were correctly diagnosed. 2 cases were correctly diagnosed by FNAC as Suspicious lesions -follicular neoplasm. Out of other 11 cases diagnosed by FNAC, 7 were follicular carcinoma and 1 was diagnosed as Papillary carcinoma (malignant neoplastic) and 3 were medullary carcinoma on histology.

Table 1: Comparison of FNAC and Histopathological diagnosis of Thyroid lesion

Crosstabulation of FNAC and Histopathology results											
		FNAC									Total
		colloid goitre	adenomatoid goiter	thyroglossal cyst	hashimoto thyroiditis	suspicious for follicular neoplasm	follicular neoplasm	papillary carcinoma	medullary carcinoma	anaplastic carcinoma	
Histology	colloid goitre	17	3	0	0	0	0	0	0	0	20
	adenomatoid goitre	4	8	0	0	0	0	0	0	0	12
	thyroglossal cyst	0	0	2	0	0	0	0	0	0	2
	hashimoto thyroiditis	0	0	0	5	0	0	0	0	0	5
	follicular adenoma	3	1	0	0	2	2	0	0	0	8
	follicular carcinoma	7	0	0	3	7	8	0	0	0	25
	papillary carcinoma	0	0	0	0	1	3	10	0	0	14
	medullary carcinoma	1	0	0	0	3	2	2	1	0	9
	anaplastic carcinoma	0	0	0	0	0	1	0	1	1	3
Total		32	12	2	8	13	16	12	2	1	98

10 cases (2 Follicular adenoma and 8 Follicular carcinoma) were correctly diagnosed as Follicular neoplasms on FNAC. Out of the other 6 cases diagnosed by FNAC, 1 was Anaplastic carcinoma and 3 Papillary carcinoma and 2 Medullary carcinoma on histology. Out of 12 cases of Papillary carcinoma on FNAC, 10 were proved to be Papillary carcinoma and rest of 2 were medullary carcinoma on histology. 1 Medullary carcinoma and 1 Anaplastic carcinoma was correctly diagnosed by FNAC.

Table 2 : Overall comparison of FNAC and Histopathological diagnosis of different benign and malignant Thyroid lesions .

(a)-FNAC DIAGNOSIS		HISTOPATHOLOGICAL DIAGNOSIS	
		Benign	Malignant
Benign	54	43	11
Malignant	15	00	15
Total	69	43	26

(b) FNAC DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	
	Malignant	Benign
Positive 15	15 (TP)	00 (FP)
Negative 54	11 (FN)	43 (TN)
Total 69	26	43

Out of 15 positive (Malignant) lesions diagnosed by FNAC, all were True positive; and out of 54 negative (Benign) lesions diagnosed by FNAC, 43 were True negative and 11 were False negative cases. Out of 98 cases, 29 cases were within suspicious of follicular neoplasm and follicular neoplasm which was excluded from calculation. So, on the basis of these observations, we found out various parameters for evaluation of FNAC diagnosis in cases of thyroid lesions (considering the histopathological diagnosis as the gold standard).1). Sensitivity = TP/TP + FN X100 = 15/15 + 11 X100 = **57.69%**

2). Specificity = TN/TN+FP X 100 = 43/43 + 0 X 100 = **100%**

3). Predictive value of Positive test = TP/TP + FP X 100 =15/15+0 X100 = **100%** 4). Predictive value of Negative test =TN/TN+FNX100 =43/43+11X100= **79.63%** 5). Accuracy rate= TP + TN/TP +TN + FP + FN X 100=15+43/69 X100= **84.06%**

6). Percentage of False Positive = FP/FP + TN X 100 = 0/0 + 43 X 100 = **0%**

7). Percentage of False Negative = FN/FN + TP X 100 =11/11+15X100 = **42.31%**

Morphometric analysis was then carried out on FNAC slides to see whether it aided pre- operative FNAC diagnosis and the MND and MNA were calculated. After morphometry, taking the 95% confidence limit into consideration, the cases were re-arranged according to cytological and histopathological correlation.

Table 3: Comparison of Morphometry assisted Mean nuclear diameter aided FNAC and Histopathological diagnosis of Thyroid lesions

Crosstabulation of Morphometry assisted mean nuclear diameter aided FNAC results with Histopathology diagnosis											
		Mean assisted diameter aided FNAC results									Total
		colloid goitre	adenomatoid goiter	thyroglossal cyst	hashimoto thyroiditis	suspicious for follicular neoplasm	follicular neoplasm	papillary carcinoma	medullary carcinoma	anaplastic carcinoma	
histology	colloid goiter	16	3	0	0	1	0	0	0	0	20
	adenomatoi d goitre	4	8	0	0	0	0	0	0	0	12
	thyroglossa l cyst	0	0	2	0	0	0	0	0	0	2
	hashimoto thyroiditis	0	0	0	5	0	0	0	0	0	5
	follicular adenoma	0	0	0	1	6	1	0	0	0	8
	follicular carcinoma	1	0	0	7	9	8	0	0	0	25
	papillary carcinoma	0	0	0	0	0	0	14	0	0	14
	medullary carcinoma	1	0	0	0	0	6	0	2	0	9
	anaplastic carcinoma	0	0	0	0	0	0	1	1	1	3
Total		22	11	2	13	16	15	15	3	1	98

Table 4 (a) shows that data changed significantly after morphometry (Morphometry assisted mean nuclear diameter aided FNAC). 47 cases were benign and 51 cases were malignant as proved by histopathology; but it was found that on **Morphometry assisted mean nuclear diameter** of FNAC, 48 cases were benign and 19 cases were malignant. Rest of 31 cases,16 cases were in the group of suspicious of follicular neoplasm and 15 cases were in the group of follicular neoplasm which was excluded from calculation.

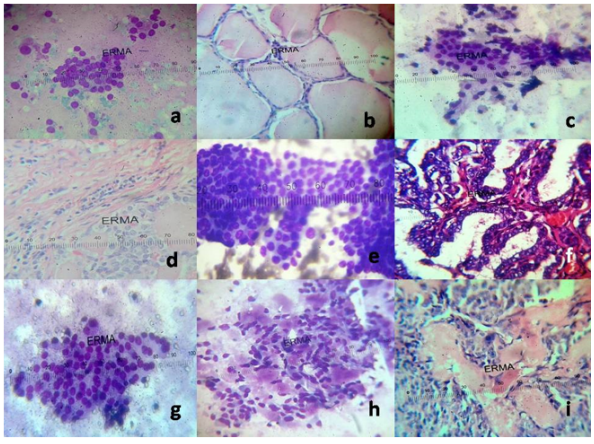
Table 4 (b) shows that out of 19 positive (Malignant) lesions diagnosed by FNAC, all were True positive; and out of 48 negative (Benign) lesions diagnosed by FNAC, 39 were True negative and 9 were False negative cases.Table 4: Morphometry aided comparison of FNAC and Histo diagnosis of different benign and malignant Thyroid lesions .

Table 4 : Morphometry assisted comparison of FNAC and Histopathological diagnosis of different benign and malignant Thyroid lesions .

(a). FNAC DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	
	Benign	Malignant
Benign 48	39	09
Malignant 19	00	19
Total 67	39	28
(b). FNAC DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	
	Malignant	Benign
Positive 19	19 (TP)	00 (FP)
Negative 48	09 (FN)	39 (TN)
Total 67	28	39

1. Sensitivity = TP/TP + FN X100 = 67.86%
2. Specificity = TN/TN+FP X 100 = 100%
3. Predictive value of Positive test = TP/TP + FP X 100 = 100%
4. Predictive value of Negative test = TN/TN + FN X 100 = 81.25%
5. Accuracy rate = TP + TN/TP +TN + FP + FN X 100 = 86.57%
6. Percentage of False Positive = FP/FP + TN X 100 = 0%
7. Percentage of False Negative = FN/FN + TP X 100 = 32.14%

Fig.1 (a&b)- FNAC & Histology of Colloid goiter.
(c&d)- FNAC & Histology of Hashimoto thyroiditis.
(e&f)- FNAC & Histology of Papillary carcinoma.
(g)- FNAC of Follicular neoplasm.
(h&I)- FNAC & Histology of Medullary carcinoma



DISCUSSION:

Till date Fine Needle Aspiration or FNA is considered as the gold standard for screening all thyroid nodular lesions. But it has got its own share of fallacies and pit-falls. Sample adequacy and accuracy is highly dependent upon the skills of the pathologist performing the test. Thyroid being an endocrine organ is highly vascular. Therefore the aspirate tends to be haemorrhagic leading to dilution of the aspirate even in the hands of trained professionals and more so when performed by clinicians and radiologists. Also, many a time lesions which have undergone cystic changes are difficult to diagnose as follicular cells are dispersed and are few and far between. Follicular neoplasms are difficult to interpret. Even follicular variant of papillary carcinomas add to the woes of the pathologists. Many a benign lesion also tend to be confusing as they pose a similar picture as that of carcinoma. Morphometry may complement cytological diagnosis and provide useful information. The potential significance of this technique is to distinguish between benign, borderline and malignant lesions, for objective grading of invasive tumors, prediction of prognosis and therapeutic response. Morphometry has been described for more than a century because the histological characteristics of normal and abnormal cells have been used as a measure of prognosis and as a way of predicting the cause of the disease. While adaptation of quantitative morphometric analysis as a clinical tool has been discussed in the literature for over 30 years, the usefulness of this approach has helped in cytological grading of breast lesions, but has not yet received widespread acceptance in thyroid lesions owing to l i m i t e d references and subjectivity. [6-11]

In our study, we evaluated 98 cases among them 32 cases were colloid goiter,12 were adenomatoid goiter, 8 cases were hashimoto thyroiditis, 2 cases were thyroglossal cyst, 29 cases were suspicious of follicular neoplasm and follicular neoplasm, 12 were papillary carcinoma, 2 cases of medullary carcinoma and 1 cases of anaplastic carcinoma. 2 cases were unsatisfactory for diagnosis and lost to follow-up. In the present series, 32 cases (33%) were diagnosed as Colloid goitre by FNAC. Pearse (1972) showed that out of 452 thyroid nodules, 45.5% were goitres.[12] Lowhagen et al (1979)obtained benign goitres in 153 cases (37.1%) in their series of 412 thyroid lesions.[13]

In the present study, the sensitivity and specificity for diagnosing thyroid neoplasia (both adenoma and malignancy) by FNAC was **57.69** and **100** respectively.The accuracy rate was **84.6%.** Prinz R A et al (1983) evaluated the overall sensitivity and specificity of FNA in diagnosing thyroid neoplasia (carcinoma or adenoma) to be 88% and 80% respectively.[14] After morphometry, in this study all the data improved substantially. The sensitivity became **67.86%**, the specificity was found to be **100%** and the accuracy rate improved to **86.57%.**

CONCLUSION:

FNA of the thyroid remains the safest and most cost-efficient manner in which to stratify thyroid nodules for surgical excision. The Bethesda System has standardized reporting nomenclature for thyroid FNA that corresponds with specific cytomorphologic criteria and risk of malignancy. Morphometric analysis in case of thyroid lesions is of definite benefit to the patient with regard to the future management as it aids an early pre-operative diagnosis with a high rate of accuracy. It may be concluded that morphometric analysis in case of thyroid lesions is of definite benefit to the patient with regard to the future management as it aids an early pre-operative diagnosis with a high rate of accuracy.

REFERENCES:

1. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: The Whickham survey. ClinEndocrinol (Oxf) 1977;7:481–93.
2. Hamburger JI, Husain M, Nishiyama R, et al. Increasing the accuracy of fine needle biopsy for thyroid nodules. Arch Pathol Lab Med 1989; 113:1035–41.
3. Boey J, Hsu C, Collins RJ. False negative errors in fine needle aspiration biopsy of dominant thyroid nodules: a prospective follow-up study. World J Surg 1986; 10: 623-30.
4. Klencka D S, Klencki M , Sporny S, Lewinski A, Karyometric Analysis in the Cytological Diagnosis of Thyroid Lesions. Analyt Quant Cytol Histol.1997;19:507-513.
5. Collan Y, Torkkeli T, Pesonen E, Jantunen E, Kosma VM. Application of morphometry in tumor pathology. Analytical and quantitative cytology and histology/the International Academy of Cytology [and] American Society of Cytology. 1987 May;9(2):79-88.
6. Nagashima T, Suzuki M, Oshida M, Hashimoto H, Yagata H, Shishikura T, et al. Morphometry in the cytological evaluation of thyroid follicular lesions. Cancer.1998;84:115-8
7. Dina R, Capitanio A, Damiani S. A morphometric analysis of cytological features of tall cell variant and classical papillary carcinoma of the thyroid. Cytopathology.2000;11:124- 8.
8. Nagashima T, Suzuki M, Nakajima N. Cytologic morphometric approach for the prediction of lymph node involvement in papillary thyroid cancer. Anal Quant Cytol Histol.1997;19:49-54.
9. Baloch ZW, Fleisher S, Li Volsi VA, Gupta PK .Diagnosis of follicular neoplasm : A gray zone in needle aspiration cytology. Diagn cytopathol.2002;26:41-4.
10. Sakamoto A, Kasai N, Sugano H. Poorly differentiated carcinoma of thyroid: A clinic pathology entity for a high risk group of papillary and follicular carcinomas. Cancer.1983;52:1849-55.
11. Slowinska-Klencha D, Klenchi M, Sporny S, Lewinski A. Size of thyrocyte nuclei in aspirates from follicular adenomas: correlation with patients, ages. Acta Cytol.1996;40:414-6.
12. Pearse A G E, Ewen S K B, Polak H. Virchow's Arch., Alat. B. Zellpath, 1972; 10: 93.
13. Lowhagen T, Granberg P O. Surg. Clin. Norht Am., W B Saunders Company, London, 1979: 59: 3-18.
14. Prinz R A, O'Morchoe P J, Barbato A L et al. Fine needle aspiration biopsy of thyroid nodules. Ann Surg. 1983 Jul; 198 (1): 70-73.