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Original Article

Study of nuclear morphometry in the cytological evaluation of thyroid neoplasms

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ABSTRACT

Background: Nuclear morphometry is purported to allow better differentiation between various thyroid lesions beyond what is offered by conventional cytology. The present study was conducted to evaluate the nuclear morphometric parameters in fine needle aspirates of thyroid neoplasms and to study their role in differentiating benign and malignant thyroid neoplasms.

Materials and Methods: Nuclear morphometric parameters of all the patients presenting with thyroid swelling and undergoing fine needle aspiration cytology (FNAC) and belonging to Category III, IV, V and VI of the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) were studied.

Results: Out of total 60 cases, maximum (21) were noted from category IV which is follicular neoplasm or suspicious for follicular neoplasm. The sensitivity of FNAC for the diagnosis of thyroid lesions in the present study was observed at 87.71%. The nuclear morphometry parameters – short axis diameter, long axis diameter, nuclear area and perimeter showed significant difference between benign and malignant lesions (p value <0.001). The mentioned nuclear morphometric parameters were observed to be the highest in anaplastic carcinoma followed by papillary carcinoma and medullary carcinoma, and lowest in follicular neoplasms. Significant difference was observed in the nuclear parameters of follicular adenoma and follicular carcinoma.

Conclusions: Nuclear morphometry can help in distinguishing between benign and malignant thyroid lesions, particularly in diagnosis of indeterminate smears where there is disparity in diagnosis on conventional cytology, especially in differentiating follicular neoplasms.

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INTRODUCTION

Thyroid nodules are a common clinical entity during clinical examinations. The worldwide prevalence of palpable thyroid nodule is estimated to range between 4-7% in the adult population; while it is reported to be approximately 12.2% in India.[1,2] An analysis of the surveillance, epidemiology and end results database from the United States showed tripling of thyroid cancer incidence over 35 years beginning in the year 1975.[3]

Fine needle aspiration cytology (FNAC) is a favoured minimally invasive technique for pre-operative diagnosis of nodular and diffuse lesions of the thyroid, with the purpose of diagnosing benign as well malignant lesions, thereby reducing unnecessary surgery.[4] Thyroid FNAC has been estimated to have a sensitivity between 65% and 98% and specificity between 72% and 100% for malignancy.[5] However, it has its limitations and the rate of false negatives is still relatively high. Furthermore, there are difficulties in differentiating follicular adenoma and goitre from follicular carcinoma.[5] Thus, there is a felt need for introducing additional means to improve the accuracy of cytological diagnosis.

Computed morphometric methods have been introduced with a view to improve the diagnostic yield of thyroid aspirates. Morphometry is the quantitative analysis of geometric features of structures with any dimension.[6] It has been suggested that nuclear morphometric parameters such as nuclear area and perimeter, nuclear area coefficient of variation and shape factors may allow differentiation between various thyroid lesions.[7-9] Application of morphometry to cytology would presumably be useful for the purpose of automated screening as well as accurate diagnosis of thyroid lesions including tumours.

The present study was conducted to evaluate the nuclear morphometric parameters in fine needle aspirates of thyroid neoplasms and to study their role in differentiating benign and malignant thyroid neoplasms. Correlation with histopathology findings was also studied in the cases in which surgery was undertaken.

MATERIALS AND METHODS

The prospective observational study was conducted by the department of Pathology at a tertiary care government teaching hospital in Central India over the period of 2 years 5 months (August 2018 to December 2020). All the patients presenting with thyroid swelling and undergoing fine needle aspiration cytology (FNAC) at our institute constituted the study population and were subsequently included as a participant as per the following inclusion criteria, viz. Category III, IV, V and VI of the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) (benign and malignant thyroid neoplasms on cytology).[10,11] Those belonging to Category I and II of Bethesda system (non-diagnostic and inflammatory lesions of thyroid) and those not consenting to undergo FNAC and/or not willing to participate in the study were excluded from the study.

The study was started after due approval from the Institutional Ethics Committee. All the patients were explained about the study and involved procedure prior to enrollment and were recruited as study participant only after taking written informed consent. All the participants were subjected to detailed history and clinical examination. FNAC, thyroid function tests and local ultrasonography of neck were performed amongst those noted to have thyroid swelling on clinical examination.

The major study variables included were FNAC diagnosis, nuclear morphometry parameters and histopathological diagnosis.

After staining and mounting of the slides, the smears were evaluated by an independent cytopathologist and after taking into consideration the cytological findings reporting was done according to The Bethesda System for Reporting Thyroid Cytopathology.[10,11]

All the FNAC cases classified as Bethesda III, IV, V, VI were taken up for morphometry study. Slides stained with hematoxylin and eosin (H and E) and Papanicolaou were used. Slides with well preserved and evenly spread material were preferred. The slides were viewed under a digital microscope and the representative images were captured and were opened in the image-based software MICAM (Microscope Image Capture And Measurement) 2.0. An average of 5 microscopic fields at magnification 400x were captured for each case. Fifty non-overlapping nuclei were selected and outlined. Calibration of the system was done by using RBC as the reference cell and also with the help of micrometer scale before measurements performed in the software. Long axis diameter and small axis diameter were measured using the image software system. These parameters were tabulated in excel sheets and were later used to calculate the other two parameters, viz. nuclear area and perimeter. The nuclear parameters in different cases were studied and cut offs to differentiate between benign and malignant cases were calculated accordingly.

Cases where surgery was performed, cytological diagnosis given according to the Bethesda classification was correlated with the histopathological diagnosis.

Statistical analysis:

The data was collected, compiled and analyzed using EPI info (version 7.2) statistical software package. The qualitative variables were expressed in terms of percentages. Normality of the data was tested using Kolmogorov Smirnov test. The quantitative variables were expressed in terms of mean and standard deviations for normal data. The difference between means of 2 groups was tested using student t test and for more than 2 groups using ANOVA. Difference between two proportions was analyzed using chi square or fisher exact test. All the analyses were 2 tailed and the significance level was set at 0.05.

RESULTS

A total of 60 eligible cases undergoing FNAC were included for the final analysis. These cases were diagnosed on cytology according to the Bethesda classification III, IV, V, VI; which includes Atypia of Undetermined Significance

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or Follicular Lesion of Undetermined Significance (III), Follicular Neoplasm or Suspicious for a Follicular Neoplasm (IV), Suspicious for Malignancy (V) and Malignant Lesions (VI). Nuclear parameters of all these 60 cases were studied and tabulated. Out of the 60 patients, 57 (95%) patients underwent surgery and histopathological diagnosis was done which was correlated with the cytological diagnosis.

The mean age of participants was 46.7 ± 16.5 years, with 63.4% of them being more than 40 years old. Majority of the patients were females (48, 80%). The commonest presenting complaint was swelling in the neck (98.33%), followed by difficulty in breathing (20%), difficulty in swallowing (10%) and loss of weight in 5% of cases. On clinical examination, the tumour size was <5 cm^2 in 22 (36.67%), 5-10 cm^2 in 18 (30%) and >10 cm^2 in 20 (33.33%) patients. Majority of the lesions (66.67%) were located in the right lobe of thyroid gland, with 30% lesions located in the left lobe and 3.33% being bilateral; as per the ultrasonography findings. Thirteen cases (21.67%) had presented with cervical lymphadenopathy and 7 (11.67%) patients had established metastasis. **(Table 1)**

Table 1: Demographic and clinical parameters of the study participants (n= 60)

	Parameter	Number	Percentage
Age	< 20 years	3	5.00%
	21-30 years	4	6.67%
	31-40 years	15	25.00%
	41-50 years	11	18.33%
	51-60 years	10	16.67%
	61-80 years	17	28.34%
Gender	Female	48	80.00%
	Male	12	20.00%
Chief Complaint	Local swelling	59	98.33%
	Breathing difficulty	12	20.00%
	Swallowing difficulty	6	10.00%
	Weight loss	3	5.00%
Tumour Size	<5 cm2	22	36.67%
	5 to 10 cm2	18	30.00%
	>10 cm2	20	33.33%
Laterality of thyroid lesion	Right lobe	40	66.67%
	Left lobe	18	30.00%
	Bilateral	2	3.33%

In the present study, The Bethesda System for Reporting Thyroid Cytopathology was adopted for further categorisation. Category 3 had 19 cases, out of which 10 cases were atypia of undetermined significance and 9 cases of follicular lesion of undetermined significance. Category 4 had 21 cases, out of which 20 cases were follicular neoplasm and one case suspicious for hurtle cell neoplasm. Category 5 had 10 cases, out of which 8 cases were suspicious for papillary carcinoma and 2 cases suspicious for medullary carcinoma. Category 6 had 10 cases, of which 5 cases were papillary carcinoma, 1 case of medullary carcinoma, 3 cases of anaplastic carcinoma and one case of recurrence of follicular carcinoma. **(Table 2)**

Table 2: Distribution of the cases based on Bethesda classification (FNAC) (n= 60)

Bethesda classification (FNAC)	Frequency
Category III: Atypia of undetermined significance or follicular lesion of undetermined significance	**19 (31.66%)**
Atypia of undetermined significance	10
Follicular lesion of undetermined significance	9
Category IV: Follicular neoplasm or suspicious for follicular neoplasm	**21 (35.00%)**
Follicular neoplasm	20
Suspicious for hurthle cell neoplasm	1
Category V: Suspicious for Malignancy	**10 (16.67%)**
Suspicious for papillary carcinoma	8
Suspicious for medullary carcinoma	2
Suspicious for metastatic carcinoma	0
Suspicious for lymphoma	0
Other	0
Category VI: Malignancy	**10 (16.67%)**
Papillary carcinoma	5
Medullary carcinoma	1
Anaplastic carcinoma	3
Recurrence of follicular carcinoma	1

Out of total 60 cases, thyroid profile results were available in all cases; of which majority were euthyroid (54, 90%), followed by 5 hyperthyroid cases (8.4%) and a solitary patient having hypothyroidism. In the Bethesda categories, most of the patients were euthyroid. Category III (AUS) had three hyperthyroid cases. Category IV had one hypothyroid and two hyperthyroid case. All the cases in Category V and Category VI were euthyroid.

The ultrasonography findings showed majority of the 60 lesions to be hypo-echoic (36, 60.00%), 20 of which belonged to Bethesda category V and VI (suspicious of malignancy and malignant). Fourteen cases (23.33%) were iso-echoic, among which 7 cases belonged to category IV. Eight cases were hyperechoic, which included four cases each from category III and IV. Remaining two cases were anechoic.

Among 24 cases which were reported as benign on histology, 5 cases (20.83%) were colloid goitre and 19 cases (79.17%) were follicular adenoma. Among 33 malignant cases, 3 cases (9.09%) were anaplastic carcinoma, 11 cases (33.33%) were follicular carcinoma, 3 cases (9.09%) were medullary carcinoma and 16 cases (48.48%) were papillary carcinoma. Among malignant cases, maximum were of papillary carcinoma. **(Table 3)**

Table 3: Distribution of the cases according to histopathology

Histopathology	Frequency	Percentage
Benign	**24**	**-**
Colloid goitre	5	20.83
Follicular adenoma	19	79.17
Malignant	**33**	**-**
Anaplastic carcinoma	3	9.09
Follicular carcinoma	11	33.33
Medullary carcinoma	3	9.09
Papillary carcinoma	16	48.48

Out of 60 cases histopathological correlation was possible in 57 cases (90%). In three cases belonging to AUS category histopathological correlation was not possible. In category III, 14 cases were positively correlated. Out of the 14 cases in category III, 9 were diagnosed as follicular adenoma on histopathology, 4 cases were of follicular carcinoma and one case was of papillary carcinoma. Two cases were misdiagnosed, which turned out to be colloid goitre on histopathology. In category IV, 21 cases which were diagnosed as follicular neoplasm were correlated on histopathology. Eight cases were diagnosed as follicular adenoma on histopathology and six cases were diagnosed as follicular carcinoma. Four cases were diagnosed as Follicular variant of papillary thyroid carcinoma. One case of Suspicious of Hurthle cell neoplasm on cytology was diagnosed as colloid goitre on histopathology. Two cases of follicular neoplasm were diagnosed as colloid goitre. In category V, six cases which were diagnosed as being suspicious of papillary carcinoma were confirmed on histopathology. Two cases belonging to the same category were diagnosed as follicular adenoma. Two cases which were diagnosed as suspicious of medullary carcinoma were confirmed on histopathology. In category VI, 5 cases of papillary carcinoma were diagnosed as the same on histopathology. Three cases of anaplastic carcinoma were confirmed to be the same on histopathology. A case of medullary carcinoma was confirmed as medullary carcinoma on histopathology. **(Table 4)**

Table 4: Correlation of cytological diagnosis with histopathology (n= 60)

Bethesda category	Cytological diagnosis	Histological diagnosis	Frequency	Remarks
Category III	AUS	FA	2	**True Positive (n= 50)**
	AUS	FC	2	
	AUS	PC	1	
	FLUS	FA	7	
	FLUS	FC	2	
Category IV	FN	FA	8	
	FN	FC	6	
	FN	FVPTC	4	
Category V	S.MED	MED	2	
	S.PAP	PAP	6	
Category VI	PC	PC	5	
	AC	AC	3	
	MED	MED	1	
	RECURRANCE- FC	FC	1	
Category III	AUS	CG	2	**False Positive (n= 7)**
Category IV	FN	CG	2	
	FN/HCN	CG	1	
Category V	S.PAP	FA	2	

**AUS- Atypia of Undetermined Significance, FA- Follicular Adenoma, FC- Follicular Carcinoma, PC- Papillary Carcinoma, FN- Follicular Neoplasm, FVPTC- Follicular Variant of Papillary Thyroid Carcinoma, S.MED- Suspicious for Medullary Carcinoma, MED- Medullary Carcinoma, AC- Anaplastic Carcinoma, CG- Colloid Goitre, HCN- Hurthle Cell Neoplasm, S.PAP-Suspicious for papillary carcinoma*

The sensitivity of FNAC as a diagnostic tool was observed to be 87.71%. Specificity and diagnostic accuracy of FNAC could not be calculated as category I and II were excluded from the study and true negative and false negative cases were not available for calculations.

In the present study it was observed that in category three, mean short axis diameter was 4.95µm with a standard deviation of 0.68, the mean long axis diameter was 5.37µm with a standard deviation of 0.73. The mean area was 84.88 μm^2 with a standard deviation of 23.67 and mean perimeter was 32.42µm with a standard deviation of 4.41. In category four, from the observed parameters mean short axis diameter was 5.21µm with a standard deviation of 1.11, the mean long axis diameter was 5.65µm with a standard deviation of 1.12. The mean area was 96.02 μm^2 with a standard deviation of 40.65 and mean perimeter was 32.12µm with a standard deviation of 6.96. In category five, mean short axis diameter was 7.05µm with a standard deviation of 1.56, the mean long axis diameter was 7.53µm with a standard deviation of 1.65. The mean area was 175.98 μm^2 with a standard deviation of 72.29 and mean perimeter was 45.79µm with a standard deviation of 10.11. In category six, mean short axis diameter was 7.77µm with a standard deviation of 2.16, the mean long

axis diameter was 8.30µm with a standard deviation of 2.22. The mean area was 216.08 µm^2 with a standard deviation of 113.59 and mean perimeter was 50.50µm with a standard deviation of 13.74. The details of nuclear Morphometry parameters in individual lesions across various TBSRTC categories are as given below. **(Table 5)**

Table 5: Nuclear Morphometric parameters across TBSRTC categories and individual lesions

Bethesda classification (FNAC)	Nuclear Morphometry							
	Short axis diameter		Long axis diameter		Area		Perimeter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Category III	**4.95**	**0.68**	**5.37**	**0.73**	**84.88**	**23.67**	**32.42**	**4.41**
Atypia of undetermined significance	5.03	0.73	5.47	0.84	88.14	26.21	33.00	4.92
Follicular lesion of undetermined significance	4.86	0.66	5.26	0.61	81.26	21.43	31.77	3.96
Category IV	**5.21**	**1.11**	**5.65**	**1.12**	**96.02**	**40.65**	**34.12**	**6.96**
Follicular neoplasm	5.26	1.11	5.71	1.12	97.95	40.81	34.47	6.96
Suspicious for hurthle cell neoplasm	4.20	-	4.50	-	59.37	-	27.34	-
Category V	**7.05**	**1.57**	**7.53**	**1.65**	**175.98**	**72.29**	**45.79**	**10.11**
Suspicious for papillary carcinoma	7.33	1.60	7.81	1.70	187.55	5.22	47.58	10.37
Suspicious for medullary carcinoma	5.75	0.07	6.25	0.21	112.92	5.22	37.72	0.90
Suspicious for other malignancy	-	-	-	-	-	-	-	-
Category VI	**7.77**	**2.16**	**8.30**	**2.22**	**216.08**	**113.6**	**50.50**	**13.74**
Papillary carcinoma	6.78	1.56	7.30	1.70	162.16	73.00	44.24	10.26
Medullary carcinoma	6.40	-	6.90	-	138.73	-	41.79	-
Anaplastic carcinoma	10.47	0.45	11.03	0.15	362.93	20.55	67.56	1.87
Recurrence of follicular carcinoma	6.00	-	6.50	-	122.52	-	39.28	-

The specific comparison of nuclear morphometry in cases of follicular adenoma and follicular carcinoma was also undertaken. The difference between the nuclear parameters of follicular adenoma and follicular carcinoma was found to be statistically significant. (P <0.001). **(Table 6)**

Table 6: Comparison of nuclear morphometry of follicular adenoma and follicular carcinoma

Disease Entity	Nuclear Morphometry							
	Short axis diameter		Long axis diameter		Area		Perimeter	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Follicular adenoma	4.57	0.27	4.99	0.17	71.79	6.45	30.06	1.32
Follicular carcinoma	5.78	0.55	6.27	0.51	114.69	18.06	37.89	2.44
P-value	<0.001	-	<0.001	-	<0.001	-	<0.001	-

DISCUSSION

In the present study, nuclear parameters of 60 cases of thyroid neoplasms diagnosed on cytology were studied. The age and gender distribution of the cases was similar to studied previously. The commonest presenting complaint was swelling in the anterior aspect of neck (98.33%), which was similar to the observed in study by Sothy k et al.[12] On clinical examination, the tumour size was <5 cm^2 in 22 (36.67%) cases, which is consistent with the findings of Aiad H et al.[7] Two third of these lesions were located in right lobe of thyroid gland (66.67%).

Majority of patients in the present study were euthyroid (54, 90%) and most of those with altered thyroid profile belonged to benign category, similar to the observations of Thakor et al.[13] Majority of the cases (36) showed hypo-echogenicity on ultrasonography and a significant proportion (20) belonged to Bethesda criteria V and VI (malignant cases), a finding similar to the study by Maia F et al.[14]

With respect to the distribution of cases in cytology according to Bethesda classification, maximum cases (21) were noted from category IV which is follicular neoplasm or suspicious for follicular neoplasm. Previous similar studies by Mondal et al, Alshaikh et al and Dhamecha M.P et al have had maximum number of cases belonging to category II followed by category IV.[15-17] The difference lies in the fact that those studies included all the categories of Bethesda; whereas the present study included only category III, IV, V and VI.

The present study revealed that on histopathological correlation the maximum number of malignant cases was found to be of papillary carcinoma followed by follicular carcinoma. This is in accordance with the studies by Anand

et al, Yashaswini et al and Melo-Uribe et al, in which papillary carcinoma was the most frequent malignant lesion observed.[18-20] Among benign cases, majority were cases of follicular adenoma followed by colloid goiter.

In cases which showed cytohistological disparity, nuclear morphometric parameters were studied. Among the seven cases, three cases of category IV and two cases of category III turned out to be colloid goitre on histopathology. On evaluating the nuclear parameters, it was observed that their parameters were lower than the cut offs set by the present study thus indicating a benign lesion. Two cases of category V, on histopathology were diagnosed to be follicular adenoma. The morphometry parameters in these cases were observed to be closer to the values observed for follicular adenoma.

The sensitivity of FNAC for the diagnosis of thyroid lesions in the present study was observed at 87.71%, which is in line with findings of previously similar studies.[21,22] The nuclear morphometry parameters – short axis diameter, long axis diameter, nuclear area and perimeter show significant difference between benign and malignant lesions (p value <0.001). This is in accordance with studies by Aiad H et al, Yashaswini R et al and Khatri P et al et al.[7,19,23] In the present study, although significant difference is observed in the nuclear parameters between benign and malignant lesions, discordance is observed in the observed nuclear parameters as compared to the other studies which could be attributed to number of nuclei measured, sample size, staining preferences and various morphometric softwares used in those studies.

The mentioned nuclear morphometric parameters were observed to be the highest in anaplastic carcinoma followed by papillary carcinoma and medullary carcinoma, with them being the lowest for follicular neoplasms. Very similar observations were previously reported by Priya et al, while Deka et al in their study had observed anaplastic thyroid carcinoma and medullary thyroid carcinoma to have the highest and lowest nuclear diameter respectively amongst the malignant cases.[24,25] Wright et al also had reported nuclear parameters of papillary carcinoma higher than follicular neoplasms.[8]

In the the specific comparison of nuclear morphometry in cases of follicular adenoma and follicular carcinoma, significant difference was observed in the nuclear parameters of follicular adenoma and follicular carcinoma in the present study, the inference being in favour of nuclear morphometry being useful in differentiation between these lesions. In a study by Tseleni-Balafouta et al**,** two morphometric variables, nuclear area and major axis length of the nucleus were studied in follicular lesions of thyroid and statistically significant differences were reported between carcinomas and adenomas and it was concluded that nuclear morphometry by computerized image analysis can be used as an additional tool in the differential diagnosis of thyroid follicular lesions in cytologically diagnosed FNA smears.[26] Rout et al had studied the diagnostic value of qualitative and quantitative variables in thyroid lesions and found quantitative variables to be higher in follicular carcinoma than follicular adenoma.[27]

In the present study, various cut offs for nuclear morphometry parameters were obtained by studying the different thyroid lesions to differentiate between benign and malignant lesions. However, the cut off values of present study and various previous studies could not be compared due to different softwares used to calculate the nuclear parameters, which emphasizes the need to standardize the software system to obtain more uniform values.

CONCLUSIONS

Nuclear morphometry can help in distinguishing between benign and malignant thyroid lesions, which is at times difficult by cytology alone. It can certainly help aid in diagnosis of indeterminate smears where there is disparity in diagnosis. Lastly, nuclear morphometry studies are particularly promising as an aide to cytology in differentiating follicular neoplasms.

Conflict of Interest: None

REFERENCES

1. Patil RS, Rashmi KP, Andola SK, Viral L, Mallikarjun B. Efficacy of fine needle aspiration cytology in diagnosis of lesions of thyroid and histopathological correlation. J Pub Health Med Res. 2013;1:18–23. Website
2. Unnikrishnan AG, Kalra S, Baruah M, Nair G, Nair V, Bantwal G, Sahay RK. Endocrine Society of India management guidelines for patients with thyroid nodules: A position statement. Indian journal of endocrinology and metabolism. 2011;15(1):2. Crossref
3. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. Jama. 2006 May 10;295(18):2164-7. Website
4. Koss LG. Diagnostic cytology and its histopathologic basis.4th ed. Philadelphia: Lippincott Williams and Wilkins; 1992. p2665-708. Website
5. Flanagan MB, Ohori NP, Carty SE, Hunt JL. Repeat thyroid nodule fine-needle aspiration in patients with initial benign cytologic results. Am J Clin Pathol. 2006;125(5):698-702. Crossref
6. Meijer GA, Beliën JA, Van Diest PJ, Baak JP. Origins of... image analysis in clinical pathology. Journal of clinical pathology. 1997;50(5):365. Crossref
7. Aiad HA, Abdou AG, Bashandy MA, Said AN, Ezz-Elarab SS, Zahran AA. Computerized nuclear morphometry in the diagnosis of thyroid lesions with predominant follicular pattern. Ecancermedicalscience. 2009;3. Crossref
8. Wright RG, Castles H, Mortimer RH. Morphometric analysis of thyroid cell aspirates. Journal of clinical pathology. 1987;40(4):443-5. Crossref
9. Słowińska-Klencka D, Klencki M, Sporny S, Lewiński A. Karyometric analysis in the cytologic diagnosis of thyroid lesions. Analytical and quantitative cytology and histology. 1997;19(6):507-13. Website

10. Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. Thyroid. 2009;19(11):1159-65. Crossref

11. Baloch ZW, Cooper DS, Gharib H, Alexander EK. Overview of diagnostic terminology and reporting. The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes. 2018:1-6. Crossref

12. Sothy K, Mafauzy M, Mohamad WB, Mustaffa BE. Carcinoma of thyroid-clinical presentation and outcome. Med J Malaysia. 1991;46(3):212-7. Website

13. Thakor T, Daveshwar MR, Shah HS. Cytomorphological Study of Thyroid Lesions Using the Bethesda System for Reporting Thyroid Cytology and Its Correlation with Thyroid Function Test. Journal of Evolution of Medical and Dental Sciences. 2020;9(12):949-53. Website

14. Maia FF, Matos PS, Silva BP, et al. Role of ultrasound, clinical and scintigraphyc parameters to predict malignancy in thyroid nodule. Head Neck Oncol. 2011;3:17. Crossref

15. Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK. The Bethesda system for reporting thyroid fine needle aspirates: A cytologic study with histologic follow-up. Journal of Cytology/Indian Academy of Cytologists. 2013;30(2):94. Crossref

16. Alshaikh S, Harb Z, Aljufairi E, Almahari SA. Classification of thyroid fine-needle aspiration cytology into Bethesda categories: An institutional experience and review of the literature. Cytojournal. 2018;15. Crossref

17. Dhamecha MP, Swami SY, Valand AG. FNAC study of thyroid lesions using the Bethesda system. Trop J Pathol Microbiol. 2018;4(1):101-8. Website

18. Anand B, Ramdas A, Ambroise MM, Kumar NP. The Bethesda System for Reporting Thyroid Cytopathology: A Cytohistological Study. J Thyroid Res. 2020;2020:8095378. Crossref

19. Yashaswini R, Suresh TN, Sagayaraj A. Cytological evaluation of thyroid lesions by nuclear morphology and nuclear morphometry. Journal of Cytology. 2017;34(4):197. Crossref

20. Melo-Uribe MA, Sanabria Á, Romero-Rojas A, Pérez G, Vargas EJ, Abaúnza MC, Gutiérrez V. The Bethesda system for reporting thyroid cytopathology in Colombia: Correlation with histopathological diagnoses in oncology and non-oncology institutions. Journal of Cytology/Indian Academy of Cytologists. 2015;32(1):12. Crossref

21. Sharma R, Verma N, Kaushal V, Sharma DR, Sharma D. Diagnostic accuracy of fine-needle aspiration cytology of thyroid gland lesions: A study of 200 cases in Himalayan belt. Journal of Cancer Research and Therapeutics. 2017;13(3):451-5. Website

22. Mumtaz K, Khadim MT, Jamil U, Haider A, Ali SS, Iram S. Diagnostic accuracy of fine needle aspiration cytology in detection of thyroid carcinoma in patients with thyroid nodules using histopathology as gold standard. Pakistan Armed Forces Medical Journal (PAFMJ). 2020;70(1):118-23. Website

23. Khatri P, Choudhury M, Jain M, Thomas S. Role of morphometry in the cytological differentiation of benign and malignant thyroid lesions. Journal of cytology. 2017;34(1):1. Crossref

24. Priya SS, Sundaram S. Morphology to morphometry in cytological evaluation of thyroid lesions. Journal of Cytology/Indian Academy of Cytologists. 2011;28(3):98. Crossref

25. Deka L, Gupta S, Gupta R, Gupta K, Kaur CJ, Singh S. Nuclear morphometry and texture analysis on cytological smears of thyroid neoplasms: a study of 50 cases. The Malaysian journal of pathology. 2017;39(1):33. Website

26. Tseleni-Balafouta S, Kavantzas N, Paraskevakou H, Davaris P. Computerized morphometric study on fine needle aspirates of cellular follicular lesions of the thyroid. Analytical and Quantitative Cytology and Histology. 2000;22(4):323-6. Website

27. Rout P, Shariff S. Diagnostic value of qualitative and quantitative variables in thyroid lesions. Cytopathology. 1999;10(3):171-9. Crossref