



CYTOMORPHOLOGY OF NODULAR THYROID LESIONS : A COMPARATIVE ANALYSIS OF WET AND AIR DRIED SMEARS

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ABSTRACT FNAC of thyroid lesions have sensitivity as high as 93.4% with a positive predictive value of malignancy 98.6 % and 74.9 % specificity. Two fundamentally different methods of fixation and staining are used in FNAC: air-drying followed by a Romanowsky stain such as May Grunwalds Giemsa (MGG), Jenner-Giemsa, Wright's stain or Diff-Quik; and alcohol-fixation followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining. Combining the morphological features of various stains often improve the diagnostic accuracy. In the present study, cytoplasmic granularity, paravacuolar granules and thin colloid are very well demonstrated using Wright Giemsa stain. Cell borders and crisp nuclear features such as chromatin pattern, intranuclear inclusions are best appreciated using wet fixed smears stained with H&E and Pap stains. The cytomorphologic features of nodular thyroid lesions using multiple cytological staining techniques to enhance diagnostic sensitivity is evaluated in this study.

KEYWORDS : Cytology, Fine Needle Aspiration, Romanowsky stain, Thyroid.

Introduction

The incidence of malignancy in a solitary thyroid nodule or in a multinodular goiter is equal and about 5% in non-endemic areas (1). FNAC of thyroid lesions have sensitivity as high as 93.4% with a positive predictive value of malignancy 98.6 % and 74.9 % specificity (2). Diagnostic accuracy is important in thyroid lesions since it decides the type of thyroidectomy performed on the patient. Two fundamentally different methods of fixation and staining are used in FNAC: air-drying followed by a Romanowsky stain such as May Grunwalds Giemsa (MGG), Jenner-Giemsa, Wright's stain or Diff-Quik; and alcohol-fixation followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining. Each of these stains highlight specific sub-cellular and extra-cellular components. Hence combining the morphological features of various stains often improve the diagnostic accuracy. The cytomorphologic features of nodular thyroid lesions using multiple cytological staining techniques to enhance diagnostic sensitivity is evaluated in this study.

Materials and Methods

The study was conducted in the cytopathology Out Patient Department, Department of Tirunelveli Medical College and Hospital. Fine needle aspirate materials obtained from nodular thyroid lesions of patients attending cytology outpatient department of Tirunelveli medical college hospital, were used in this study. Study material included fine needle aspirate materials obtained from nodular thyroid lesions of 40 patients attending cytology OPD. FNAC was performed using 23 G needles, after obtaining informed written consent from the patients and multiple smears were prepared. Two smears were wet fixed and stained with H&E and Pap stain. One or two smears were dry fixed and stained with Wright-Giemsa. Stained slides were observed using light microscope and analysed. The cytomorphologic features of thyroid lesions were analysed using various cytological staining techniques namely Hematoxylin and eosin stain, Papanicolou stain and Wright Giemsa. Cases were followed up and correlated with histopathology if available.

Results

TABLE 1 : DISTRIBUTION OF LESIONS

S.No	Lesion	Number of cases	Percentage
1	Nodular colloid goiter	24	60

2	Nodular colloid goiter with cystic degeneration	8	20
3	Follicular neoplasm	4	10
4	Papillary carcinoma of thyroid	3	7.5
55	Suspicious of papillary carcinoma of thyroid	1	2.5

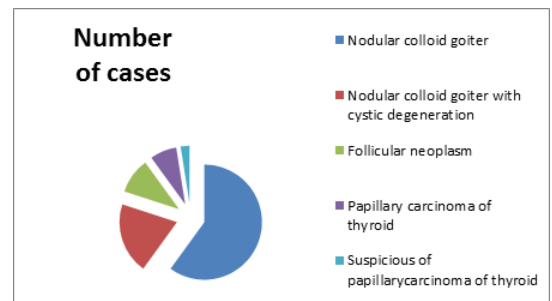


FIGURE 1 : DISTRIBUTION OF LESIONS

Histopathological correlation was available for 29 cases. A positive correlation was observed in 16 cases of nodular colloid goiter, 6 cases of nodular colloid goiter with cystic degeneration, 3 cases of follicular neoplasm and 2 cases of papillary carcinoma of thyroid. Two cases reported as nodular colloid goitre turned out to be follicular adenomas.

1. Nodular colloid goiter :

- Wright Giemsa stain : Follicular cells had pale ill defined cytoplasm and round nucleus with open chromatin. Colloid material took bluish violet colour. Thick colloid showed cracking artifacts and it was easy to identify thin colloid.
- Papanicolou stain : The thyroid follicular cells had uniform round nuclei with scanty delicate cytoplasm. Thick and thin colloid took varying shades of light greenish blue colour, pinkish colour and orangish colour. Follicular cells showed striking anisokaryosis.
- Hematoxylin and eosin stain : Thyroid follicular cells had scant

cytoplasm and round nucleus with condensed chromatin. Colloid appeared as eosinophilic material. Anisokaryosis was noted in the follicular cells. When aspirates are overly bloody, serum may be mistaken for colloid,

especially on Pap stains. This problem was overcome by using air dried smears stained with Romanowsky stains such as Wright Giemsa / MGG in which thin colloid appears as watery blue.

2. Nodular colloid goiter with cystic degeneration

- I. Wright Giemsa stain : Cyst macrophages showed dusky grayish cytoplasm and follicular cells had pale ill defined cytoplasm and round nucleus with condensed chromatin.
- ii. Papanicolou stain : Benign follicular cells and cyst macrophages were seen in the background of colloid or fluid background. Cyst macrophages showed engulfed colloid within their cytoplasm. Follicular cells showed anisokaryosis.
- iii. Hematoxylin and eosin stain: Follicular cells arranged in microfollicles and macrofollicles admixed with cyst macrophages were in the background of colloid. The cyst macrophages had vacuolated cytoplasm. Few had hemosiderin laden macrophages. Anisokaryosis was well appreciated in Pap and H & E stained smears. Engulfed colloid within the cytoplasm of cyst macrophages were well demonstrated using Pap stain. This could help in determining whether the cystic degeneration is recent or old.

3. Follicular neoplasm :

- I. Wright Giemsa stain : Thyroid follicular cells showed moderately preserved microfollicular architecture with nuclear crowding and overlapping in some foci. Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are enlarged, round to oval and contain uniformly dispersed coarse granular chromatin.
- ii. Papanicolou stain : Predominant microfollicular architecture and three dimensional clusters. The follicular cells were monomorphic with pale ill defined cytoplasm and round nucleus with smooth contour. Background was hemorrhagic and free of colloid
- iii. Hematoxylin and eosin stain: Repetitive microfollicular pattern and three dimensional clusters with uniform cell population in a hemorrhagic background. The distinction between follicular neoplasm and nodular goiter with microfollicular focus may lead to diagnostic difficulties. The amount of colloid in the background is an important feature for differentiating these two entities. Colloid was scant or absent in follicular neoplasm, whereas in case of nodular goiter there was more colloid. The presence of abundant blood in smears helped in the diagnosis of follicular neoplasm, since they are highly vascularised. Another distinguishing feature is the anisokaryosis. Anisokaryosis was observed in cases of non-neoplastic lesions of thyroid such as nodular goiter and thyroiditis. Even mild anisokaryosis was well demonstrated in wet fixed Pap stained smears.

4. Papillary carcinoma :

- I. Wright Giemsa stain : Smears showed papillary clusters and monolayered groups of thyroid follicular cells with enlarged nuclei, irregular nuclear contour and fine granular chromatin. Some of the cells showed intranuclear cytoplasmic inclusions.
- ii. Papanicolou stain : Papillary architecture is very well maintained in Pap stained smears. Nuclear crowding, overlapping and intranuclear cytoplasmic inclusions and nuclear grooves were easily demonstrated. Follicular cells with fine granular chromatin (powdery chromatin) and inconspicuous nucleoli are best appreciated in Pap stained smears.
- iii. Hematoxylin and eosin stain: Follicular cells were arranged in papillary clusters with fine granular chromatin. Nuclear crowding, overlapping and intranuclear cytoplasmic inclusions were seen. One case showed nuclear grooves in H&E stained smear.

Differential diagnosis include papillary hyperplasia in other lesions of thyroid and Hyalinising trabecular tumour. Difficulty in diagnosis arise in cases of cystic change, lymphocyte infiltrate in papillary carcinoma and follicular variant of PC, which may lead to false positive or false negative diagnosis. In those circumstances, careful inspection of nuclear features such as powdery chromatin, intranuclear cytoplasmic inclusions and nuclear grooves better appreciated using wet fixed Pap stained smears, thus aiding in improvement of diagnostic accuracy.

Discussion

Selection of an appropriate stain for FNAC is the basis of obtaining

reliable and optimal results. Minimising false negative and false positive reports is greatly dependant on type and quality of stains used. The present study analyses the cytomorphologic features of individual thyroid lesions using H&E, Pap and Wright Giemsa stains. These three stains are widely used in most cytology laboratories

Krafts K et al³ states that Romanowsky-type stains enhance cytoplasmic detail, a useful feature for determining differentiation of neoplastic cells, Romanowsky-type stains frequently show excellent granule detail Romanowsky-type stains are superior to wet fixed preparations for diagnosing hematomorphoid neoplasms, particularly in body fluid specimens. Certain characteristic features of hematomorphoid cells either not seen or are more difficult to recognize in Papanicolaou or hematoxylin and eosin stains.

The present study comprised of 24 cases of nodular colloid goiter. Literature described that colloid appear as fine film of varying colour from gray-green to rose⁴, Colloid is cyanophilic to eosinophilic and orangish when mixed with blood⁵. In PAP-stained smears, thin colloid stains pale green or orange, with cracking artifacts seen. Thick colloid appears as clumps of dark green or orange material²

In Romanowsky stains, thin colloid is recognized easily owing to its characteristic folding and cracking patterns, which imparts a "mosaic like crackling" described by Krafts, Kp et al⁶ "crazy pavement" appearance or "cracked glass" and red-violet colour⁴. Orell et al² describes blue violet colour and hyaline texture of colloid appear to be an advantage in May Grunwalds Giemsa (MGG)-stained smears and helps in distinguishing it from fibrillary collagen and deep magenta staining amyloid. In present study colloid appeared as eosinophilic in H&E stained smears. Colloid took light greenish blue colour, pinkish colour and orangish colour in Pap stained slides. In Wright Giemsa stained smears colloid took bluish violet colloid. Thick colloid showed cracking artifacts. And it was easy to identify thin colloid in air dried smears stained with Wright Giemsa stain. The present study comprised of 4 cases of follicular neoplasm. E.A Sinna et al⁷ described a case of follicular neoplasm showing atypical follicular cells with high N/C ratio and nuclear pleomorphism, arranged in three dimensional cluster with focal attempt at acinar arrangement in pap stained smears. Marluce Bibbo et al⁴ described smears of follicular neoplasm are cellular with tissue fragments showing marked crowding and overlapping. Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are round and enlarged in size in Romanowsky stained smears.

In the present study wet fixed smears stained with hematoxylin and eosin and Pap stains showed aspirates with very well preserved cellular architecture. Predominant microfollicular architecture and three dimensional clusters are seen. The follicular cells showed variable nuclear atypia. In Wright Giemsa stained smears the thyroid follicular cells showed moderately preserved microfollicular architecture with nuclear crowding and overlapping in some foci. Follicular cells had pale and poorly defined cytoplasmic limits. Nuclei are enlarged, round to oval and contain uniformly dispersed coarse granular chromatin. The present study comprised of 4 cases of papillary carcinoma of thyroid.

E.A Sinna et al⁷ described a case of papillary carcinoma showing characteristic papillary configuration. The nuclei show ground glass chromatin, intranuclear cytoplasmic inclusions and characteristic clefts in Pap stained smears. In the present study papillary architecture is very well maintained in H&E and Pap stained smears. Nuclear crowding, overlapping and intracytoplasmic nuclear inclusions are easily demonstrated using H&E and Pap stained smears. One case showed nuclear grooves in H&E stained smear. Follicular cells with fine granular chromatin (powdery chromatin) and inconspicuous nucleoli are best appreciated in Pap stained smears. Wright Giemsa stained smears showed papillary clusters and monolayered groups of thyroid follicular cells with enlarged nuclei, irregular nuclear contour, fine granular chromatin and abundant cytoplasm. Some of the cells showed intranuclear cytoplasmic inclusions.

Conclusion

Most studies have reported high accuracy rates of FNAC in the diagnosis of neoplasm and thyroiditis and it depends on stains used. In the present study, the cytoplasmic features such as cytoplasmic granularity, paravacuolar granules and thin colloid are very well demonstrated using Wright Giemsa stain. Cell borders and crisp nuclear features such as chromatin pattern, intranuclear inclusions are best appreciated using wet fixed smears stained with H&E and Pap

stains. Romanowsky-type stains provide several advantages for evaluation of routine cytological specimens including accentuation of cell and nuclear size differences, accentuation of nuclear chromatin, enhanced visibility of cytoplasmic detail, enhanced visibility of smear background elements, and superior demonstration of intercellular material. Hence the use of different methods of fixation and multiple stains was found to complement each other and aid in improving diagnostic accuracy of nodular thyroid lesions.

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