

Original Research Article

COMPARISON OF CYTOMORPHOLOGIC CHARACTERISTICS OF THYROID LESIONS UTILISING DIFFERENT CYTOCHEMICAL STAINING TECHNIQUES

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ABSTRACT

Background: To use different cytological staining techniques to examine the cytomorphic aspects of thyroid lesions.

Materials and Methods: 100 individuals with adequate aspirates participated in an experimental study carried out at the cytopathology outpatient department. The assignment was finished when the Institutional Ethical Committee granted the necessary authorization.

Results: Histopathological correlation was obtained for 25 of the 100 cases in this investigation. For seven cases of Hashimoto's thyroiditis, one case of lymphocytic thyroiditis, six cases of nodular colloid goitre, eight cases of nodular colloid goitre with cystic degeneration, seven cases of follicular neoplasm, and six cases of thyroid papillary carcinoma, histopathological linkage was available. Histopathological studies of those 25 instances showed a correlation with the cytological diagnosis. Three instances that were first diagnosed as follicular neoplasms based on cytology were later identified as follicular carcinomas.

Conclusion: A combination of several stains and different fixation procedures will boost the diagnostic accuracy of thyroid lesions. Thus, in order to decrease false positive and false negative results in fine aspiration cytology of thyroid lesions, a range of cytological staining methods can be applied, given appropriate material availability.

Keywords: Thyroid lesions, cytochemical staining, Wright Giemsa, pap stain, haematoxylin and eosin.

INTRODUCTION

Thyroid lesions are commonly observed in clinical settings. The method of fine-needle aspiration (FNA) was initially utilised by Martin and Ellis at New York Memorial Hospital for Cancer and Allied Diseases in 1930 to examine thyroid tumours at a cellular level. Thyroid fine-needle aspiration (FNA) is a widely used and proven minimally invasive diagnostic procedure for evaluating thyroid conditions. It is considered the most cost-effective and reliable method worldwide. In regions that are not endemic to the condition, the prevalence of cancer is nearly equal, at around 5%, in both a single thyroid nodule and a multinodular goiter.^[1,2]

Therefore, it is imperative to distinguish benign lesions prior to surgery in order to avoid unnecessary surgeries. The sensitivity of fine needle aspiration cytology (FNAC) in detecting thyroid lesions is as high as 93.4%, with a positive predictive value for malignancy of 98.6% and a specificity of 74.9%. Precise diagnosis is essential in thyroid lesions as it dictates the precise type of thyroidectomy to be conducted on the patient.^[3,4] FNAC utilises two separate methods for fixation and staining: air-drying with Romanowsky stains such as May Grunwalds Gimsa (MGG), Jenner-Giemsa, Wright's stain, or Diff-Quik; and alcohol-fixation followed by Papanicolaou (Pap) or hematoxylin and eosin (H&E) staining. Both systems have their own

advantages and disadvantages. Both methods are valuable as specific attributes of cells, cellular products, and stroma are more effectively demonstrated by one approach in comparison to the other. By including the morphological attributes of several stains, the accuracy of diagnosis can be improved.^[5,6]

Ultrasonography (US), thyroid function tests, antibody profiles, and fine-needle aspiration (FNA) are combined in specific situations to synergistically improve their efficacy. In recent times, various immunocytochemical and molecular markers have been employed on thyroid cytological material to improve diagnosis accuracy and offer direction for therapeutic strategies.^[7-9]

MATERIAL AND METHODS

An experimental investigation was conducted in the Department of Pathology, Government Medical College, Nandyal, Andhra Pradesh, India on 100 patients with sufficient aspirates from December 2022 to November 2023. The task was completed following the acquisition of requisite authorization from the Institutional Ethical Committee.

Inclusion Criteria

1. Fine needle aspirate materials acquired from thyroid lesions of patients

Exclusion Criteria

1. Non cooperative patient
2. Insufficient information on Fine Needle Aspiration (FNA).

RESULTS

Nine cases fell into category IV (follicular neoplasm), six cases fell into category V (suspicious for malignancy), which is suspicious for thyroid papillary carcinoma, and nine cases fell into category VI (malignant). Of the 100 cases, 29 cases had Hashimoto's thyroiditis, 9 cases had lymphocytic thyroiditis, and 29 cases had nodular colloid goitre. [Table 1]

Lymphocytic thyroiditis, or Hashimoto's thyroiditis

Wright Giemsa stain

The cells had a slight enlargement. The Hurthle cells exhibited a nucleus that was positioned either centrally or eccentrically, characterised by finely packed chromatin. Additionally, they displayed abundant cytoplasm with small granules that had an affinity for both acid and basic dyes. Furthermore, there was a slight variation in the size of the nuclei. The follicular cells had a round nucleus with

densely packed chromatin and a pale, indistinct cytoplasm. Evidence clearly demonstrates that lymphocytes and plasma cells exhibit polymorphism.

Papanicolaou Stain

Pap stained smears revealed the presence of Hurthle cells characterised by a nucleus positioned centrally or eccentrically, with thin chromatin, abundant deep eosinophilic cytoplasm, and evident anisokaryosis. In three instances, the Pap stain detected intranuclear inclusions. The cytoplasm of follicular cells exhibited indistinct boundaries, while their nucleoli seemed diminutive and spherical, characterised by delicate chromatin. Lymphocyte infiltration was seen in the background.

Stain hematoxylin and eosin

The Hurthle cells displayed a nucleus that was either centrally or eccentrically positioned, with chromatin that was tightly packed. The cytoplasm was abundant and stained eosinophilic, and there was evident variation in the size of the nuclei (anisokaryosis) in the H&E stained smears. The follicular cells exhibited an absence of distinct spherical nucleus, cytoplasm, and condensed chromatin. Lymphocyte infiltration was seen in the background.

Wright Giemsa stain

The exhibition showcased the cytoplasmic granularity of Hurthle cells, together with the diverse population of lymphocytes and plasma cells. The anisokaryosis of Hurthle cells was readily apparent when using the Pap stain. In three instances, the Pap stain detected intranuclear inclusions.

The presence of stripped follicular cell nuclei, which mimic lymphocyte nuclei, makes differential diagnosis challenging. The nuclei of stripped follicular cells do not have the dark-staining edge of cytoplasm that is seen in lymphocytes. Instead, they have more consistent chromatin and a thicker nuclear boundary. Lymphocytes were more distinctly discernible from the nuclei of exposed follicular cells in Romanowsky-stained smears.

Differentiating Hurthle cell neoplasm from Hashimoto's thyroiditis is crucial, and can be done by examining the cytological features of these cells. The oncocytes in thyroiditis exhibited characteristics of being big, unusual, and pleomorphic. Nevertheless, the cells in Hurthle cell neoplasm exhibit a consistent lack of variation. The utilisation of alcohol-treated Papanicolou stain was effective in identifying anisokaryosis.

Table 1: Lesions Distribution

S. No	Lesion	Number of cases	Percentage of cases
1	Hashimotos thyroiditis	26	26 %
2	Lymphocytic thyroiditis	9	9 %
3	Nodular colloid goiter	29	29 %
4	Nodular colloid goiter with cystic degeneration	13	13 %
5	Follicular neoplasm	9	9 %
6	Suspicious of papillary	6	6 %

	carcinoma of thyroid		
7	Papillary carcinoma of thyroid	8	8 %
	Total	10	100%
		0	

Table 2: Correlation of histopathological findings

S. No	Lesion	Total no. Of cases	Cases with histopathological correlation
1	Hashimotos thyroiditis	26	4
2	Lymphocytic thyroiditis	9	2
3	Nodular colloid goiter	29	7
4	Nodular colloid goiter with cystic degeneration	13	5
5	Follicular neoplasm	9	4
6	Papillary carcinoma of thyroid	8	3

DISCUSSION

Fine Needle Aspiration Currently, the cytology of thyroid nodules is a frequently used medical procedure performed on an outpatient basis. It is considered cost-effective and involves minimal invasion. The interpretation of FNAC smears is mostly influenced by two key criteria: sampling and stain quality. The pathologist's proficiency in sampling is crucial for the staining process, which relies on the specific type of stain and the method of application. To obtain precise and optimal outcomes from FNAC, it is crucial to select the appropriate stain as the initial step.^[9,10]

Jyothi K. conducted a study where we utilised H&E, Pap, and Wright Giemsa stains to analyse the cytomorphologic features of specific thyroid lesions. These three stains are essential for many cytology labs. This investigation includes a total of twenty-two patients diagnosed with Hashimoto's thyroiditis. Nguyen G-K et al. discovered sheets of follicular epithelial cells exhibiting oncocytic transformation on a Pap stained smear, combined with benign lymphoid cells.^[11]

Wet fixed smears stained with both Pap and H&E stains exhibit exceptional maintenance of the cellular structure in instances of Hashimoto's thyroiditis. The Pap stained smears showed Hurthle cells characterised by a nucleus placed in the centre and a large amount of cytoplasm that appeared deep pink under the microscope, in contrast to the H&E stained smears. The presence of Hurthle cell anisokaryosis is clearly demonstrated by the Pap stain. In three instances, the Pap stain detected intranuclear inclusions. The cells exhibited a little increased size when observed on Wright Giemsa stained slides following the process of air drying. Observation of cytoplasmic granules in Hurthle cells is evident. The cytoplasm of Hurthle cells exhibited an amphobolic coloration. The diverse populations of lymphocytes and plasma cells in Wright's samples are effectively depicted using Giemsa stain. Our findings aligned with the studies conducted by Krafts K et al. and Nguyen G-K et al.^[10,11]

Furthermore, it is crucial to take into account the choice and quality of the stain in order to minimise the occurrence of both false positive and false negative results. The present work used the Pap, Wright Giemsa, and H&E stains to analyse the

cytomorphologic features of individual thyroid lesions. These three stains are widely used in most cytology facilities. The current study reports a total of 29 instances of Hashimoto's thyroiditis. Nguyen G-K et al. observed clusters of follicular epithelial cells with oncocytic alteration interspersed with non-cancerous lymphoid cells in a Pap stained sample. Krafts K et al. state that Romanowsky-type stains frequently display exceptional granule detail and enhance cytoplasmic detail, which is beneficial for distinguishing neoplastic cell differentiation.^[11,12]

The current analysis identified a total of 29 cases with nodular colloid goitre. The literature reports that colloids can manifest as a delicate layer exhibiting a spectrum of colours, ranging from gray-green to rose. When mixed with blood, colloid takes on an orange hue and exhibits a strong affinity for staining with eosin. Colloid stains with a thin consistency exhibit a pale green or orange colour in PAP-stained smears, and can be observed with obvious cracking artefacts. Clusters of orange or dark green particles suggest the presence of a dense colloid. The thin colloid in Romanowsky stains can be readily distinguished due to its unique folding and breaking patterns. According to Krafts, Kp et al., these patterns result in a reddish-violet colour and a visual effect like a "crazy pavement" or "cracked glass". Orell et al. observed that in May Grunwalds Giemsa (MGG)-stained smears, the colloid exhibits a distinctive blue violet colour and hyaline texture.^[10,11,12] This characteristic is advantageous in distinguishing it from fibrillary collagen and amyloid, which stain with a deep magenta colour. Colloid was observed in the H&E-stained smears of the ongoing inquiry, appearing as eosinophilic. The colloid produced hues on Pap-stained slides that ranged from light greenish blue to pink and orange. The colloid in Wright Giemsa dyed smears had a bluish violet coloration.^[13,14]

Venkatesh K et al. observed that MUFPP achieved a clean background in 80% of salivary gland smears and 76.7% of thyroid lesions. When using the REAP stain, the clean background rates were 76.7% and 26.7% for salivary gland and thyroid lesions, respectively. Shinde PB and Pandit AA observed that 95% of the smears in MUFPP had a clear background. They conducted a study on four locations - lymph node, breast, thyroid, and salivary

gland - to examine the quality index (QI). Choudhary P et al. discovered that smears stained with MUFPP exhibited a clear background and improved morphology, resulting in enhanced quality indicators for breast and lymph node lesions.^[15,16,17] The colloid contained fractured artefacts. In addition, the identification of thin colloid was straightforward in air-dried smears stained with Wright Giemsa pigment. Sidawy and Costa et al. have reported that thyroid fine needle aspirates often include paravacuolar granules, which are lysosomes containing hemosiderin or lipofuscin colours. For diagnosing hematolymphoid neoplasms, Romanowsky-type stains are superior than wet fixed preparations, particularly for examining body fluid samples. Papanicolaou or hematoxylin and eosin stains may not exhibit some discernible characteristics of hematolymphoid cells, or these traits may be more challenging to observe. Hurthle cells demonstrate substantial variation in nuclear size, and occasionally, the nucleus in Pap-stained smears may contain cytoplasmic inclusions. Marluce Bibbo has noticed irregularly shaped Hurthle cells with polygonal dense granular cytoplasm, a somewhat eccentric nucleus with fine granular chromatin, and anisokaryosis in Romanowsky-stained smears.^[18,19]

In the current examination, paravacuolar granules were identified in a single case of nodular colloid goitre in a patient with documented hyperthyroidism. There were four cases of follicular neoplasm in the current study. E.A. Sinna et al. described a case of follicular neoplasm. The pap stained smears showed abnormal follicular cells with a high nucleus-to-cytoplasm ratio and irregular nuclear shape, arranged in a three-dimensional cluster with a focal attempt at acinar organisation. Marluce Bibbo et al. reported on the smears of follicular neoplasms. The cells should display a compact arrangement with overlapping tissue segments. The cytoplasmic borders of follicular cells appeared pale and poorly defined. Smears stained with Romanowsky show the presence of round nuclei that are greater in size.^[19,20]

A nuclear groove was observed in one instance while examining the H&E stained smear. Papanicolaou stained smears are optimal for visualising follicular cells, characterised by finely granulated chromatin (powdery chromatin) and inconspicuous nucleoli. The thyroid follicular cells, observed in Wright Giemsa stained smears, displayed enlarged nuclei, irregular nuclear shape, finely granulated chromatin, and expanded cytoplasm. Observation of cells containing intranuclear cytoplasmic inclusions was made.^[20,21]

The current work involved examining wet fixed smears stained with Pap and hematoxylin and eosin. The results showed hypercellular aspirates with very well-preserved cellular architecture. The presence of three-dimensional clusters and a prominent microfollicular structure can be recognised. Follicular cells exhibited variable nuclear atypia.

The thyroid follicular cells exhibited nuclear crowding and a partially preserved microfollicular structure in smears stained with Wright Giemsa. Additionally, there were areas where the cells overlapped. The cytoplasmic borders of the follicular cells were pale and indistinct. The nuclei are enlarged and have a round to oval shape. They include coarse granular chromatin that is equally dispersed. This investigation comprised four cases of thyroid papillary cancer. E.A. Sinna et al. reported a case of papillary carcinoma that displayed the characteristic papillary structure. The nuclei of Pap stained smears exhibit chromatin with a ground glass appearance, as well as intranuclear cytoplasmic inclusions and characteristic clefts.^[20-23] The current analysis reveals excellent preservation of the papillary architecture in the H&E and Pap stained smears. H&E and Pap stained smears provide a straightforward method for visualising nuclear crowding, overlapping, and intracytoplasmic nuclear inclusions. One case's H&E-stained smear had obvious nuclear grooves. The most prominent feature of Pap stained smears is the presence of follicular cells with finely granular chromatin (resembling powder) and inconspicuous nucleoli. Wright Giemsa stained smears revealed the presence of papillary clusters and monolayered groups of thyroid follicular cells. These cells had enlarged nuclei, irregular nuclear outlines, thin granular chromatin, and expanded cytoplasm. Some cells contained intranuclear cytoplasmic inclusions.^[24]

CONCLUSION

In order to eliminate the possibility of neoplasia, it is necessary to thoroughly evaluate any enlargement of the thyroid, whether it is diffuse or nodular in nature. FNAC, together with other tests, serves as the primary investigative method to avoid unnecessary operations. Based on the stains used, most research studies have demonstrated that FNAC (Fine Needle Aspiration Cytology) has good accuracy rates in identifying thyroiditis and neoplasms.

The study utilises Giemsa stain to clearly depict the cytoplasmic attributes, including cytoplasmic granularity, paravacuolar granules, and thin colloid. To effectively visualise cell borders and distinguish nuclear features such as chromatin pattern and intranuclear inclusions, it is recommended to utilise wet fixed smears that have been stained with Pap and H&E stains. The Papanicolaou stain is frequently used in cytopathology due to its distinct nuclear characteristics, such as intranuclear inclusions, chromatin pattern demonstration, and cell size similar to histopathology. Many laboratories employ hematoxylin and eosin stain due to its simplicity of application compared to Pap stain and its provision of superior nuclear characteristics.

When assessing regular cytological specimens, Romanowsky-type stains provide several advantages, including the ability to emphasise variations in cell and nuclear size, enhance nuclear chromatin visibility, improve the clarity of cytoplasmic details, display intercellular material more effectively, and enhance the visibility of background elements in the smear. Moreover, the utilisation of Romanowsky-type stains is essential in particular diagnostic scenarios when Papanicolaou stains are not particularly useful. These environments encompass the rapid evaluation of diagnostic samples, the detection of microorganisms, and the diagnosis of blood and lymphatic system tumours.

It can be inferred that the utilisation of many stains and diverse fixation procedures will synergistically enhance the precision of diagnosing thyroid lesions. Thus, in the context of fine aspiration cytology of thyroid lesions, several cytological staining procedures can be utilised, given the presence of sufficient material, in order to minimise the occurrence of both false positive and false negative results.

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Conflict of interest

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