

**Review Article**

Cell Block Preparation for Fine Needle Aspiration Cytology of Thyroid; Cost Effective Method in a Resource Poor Setting

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Abstract: Over the past thirty years thyroid tumor incidence has triplicated and it is currently 7% of a given population. However, interestingly the thyroid cancer incidence is quite low and is approximately 5-10% of the thyroid tumor patients. Expedient diagnosis of the thyroid nodules at the pre surgery stage is important to determine the tumor prognosis. A widely used diagnostic approach is the direct observation of the fine needle aspiration specimens. However, using only this method many reported cases of unnecessary surgeries and re-operative incidents due to false diagnosis at the preoperative stage have been observed. Poor cellularity, loss of tissue architecture, poor cytomorphologic features in fine needle aspiration smears leads to incorrect diagnosis. Cell block technique is a better diagnostic approach at the pre-operative stage to overcome the pitfalls in direct smears as it concentrates the cells and preserve tissue architecture. Further, multiple sections with same cellularity can be prepared from a single cell block allowing the use for more advanced techniques like immunohistochemistry and molecular diagnosis. Cell block technique strengthens the diagnosis of tumors by fine needle aspiration specimens. Combined use of cell block technique with fine needle aspiration can reach to a sensitivity of about 95-100% suggesting that every FNAC specimen should be subjected to cell block preparation whenever possible.

Keywords: Cell Block, Fine Needle Aspiration Cytology, Thyroid Tumors, Immunohistochemistry

1. Introduction

Over the past thirty years thyroid carcinoma incidence has triplicated in the United States and in other countries and continue to increase globally. According to Global Cancer Observatory of World Health Organization thyroid carcinoma

incidence is 6.7 per 10000 populations. Thyroid cancer is regarded as the fifth most common type among females worldwide accounting for an incidence of 10.2 while it is considered the second commonest type, among Sri Lankan women according to cancer incidence data. [48, 49]

As the tumor prognosis can only be predicted by prompt diagnosis of the disease condition, different diagnostic

approaches are available. These approaches can expand from routine ultrasound scanning, fine needle aspiration cytology, different serum biochemical investigations and histopathological investigations to more advanced immunohistochemistry, immunocytochemistry and molecular diagnosis.

Direct smears prepared from the fine needle aspiration is considered as a safe, reliable and accurate diagnostic method in the preoperative diagnosis of thyroid lesions [3]. FNAC is done radiology guided or by palpations. Main objective of conducting FNAC is to stratify patients with a risk of malignancy, subjecting them to surgeries and preventing unnecessary surgeries for benign nodules. However, sensitivity and specificity of this technique is low as individual cell morphologies are considered and as there is a chance of missing micro carcinomas [18]. Further, there are reported cases with false diagnosis due to overlapping of the cytological criteria and cellularity in hyperplasia. [4] In order to conduct histopathological diagnosis which is considered as the gold standard, specimens have to be collected by a surgical procedure. This is a labor intensive procedure requiring both technical and clinician's aspect and is an obstacle for expeditious diagnosis of the tumours. Unnecessary surgical removal and re-operative incidents are recorded as proper diagnosis of thyroid malignancy is determined only after histological examination of the tissues. Regardless of the method expeditious and accurate diagnosis is essential for proper patient management.

In order to make an accurate diagnosis during preoperative period cell block technique has been resorted to fort maximum use of available material. Current clinical setup utilizes this technique as this overcomes the issues generated from fine needle aspiration cytology due to poor cell concentration, poor cytomorphologic features and poor architectural insights [5]. Further, this technique helps to reduce the undetermined cases of thyroid FNAC [51].

2. Cell Block Preparation

Variety of techniques are used to prepare cellblocks with sufficient cellularity and better preservation of the cells and architecture. These cell blocks are prepared using either fine needle aspiration or needle wash. Cytology specimen scrapings can also be used for cell block preparation. Further, conventional cell blocks and cyto scraped cell blocks are equally effective in the preservation of cytomorphologic features of the cells. There is no definitive solution to be used to collect the cytology specimens. However, saline, formalin and alcohol based media are commonly used for specimen collection. For cyto scraped cell block preparation, coverslips are removed in xylene and dipped in absolute alcohol for 20 minutes followed by the scraping of the material by a high profile blade and the scraping is added to an anticoagulated tube. [6, 7]

2.1. Saline Plasma Thrombin Method

In this technique of cell block preparation needle wash or the FNA specimen is added to normal saline. This is

centrifuged at a speed of 2500rpm for a duration of 10 minutes. Subsequently the supernatant is decanted and 0.5 ml of plasma is added to the sediment. This mixture is subjected to vortex and 0.25-0.5ml of thrombin reagent is added and is allowed to form a clot trapping the cells in the specimen. The prepared cell block is fixed in formalin and used for further processing.

2.2. Plasma Thromboplastin Method

In this technique specimen is centrifuged at a speed of 1000G for 10 minutes and the deposit is taken for the cell block preparation. The deposit/ pellet is suspended in 3 drops of plasma followed by 03 drops of thromboplastin reagent. The mixture is gently suspended, and 03 drops of calcium chloride is added. Subsequently this is allowed to stand undisturbed for 15-20 minutes. 5-8ml of 10% buffered formalin is added to the cell block and allowed to fix. After fixation the cell block is kept in a tissue cassette and subjected to further processing [5]. This technique can be modified to apply in resource poor setups by omitting the centrifugation step. In this modified technique, FNAC aspirate is directly collected into 2ml of pool fresh plasma and then 2 drops of thromboplastin reagent is added. Then the formed cell block is processed as routine histologic specimens [52].

2.3. Histogel Procedure

Histogel procedure of cell block preparation is a technique which uses the formalin rinsed needle wash or FNAC specimen. This specimen is centrifuged at a speed of 2500rpm for 10 minutes. Warmed 4 drops of histogel is added to the sediment and it is allowed to cool to room temperature. The cellular pellet formed at this time is taken to tissue cassette and fixed in formalin solution used for further processing.

2.4. Improvised Ethanol Formalin Fixative Method

Aspirated material and the needle is rinsed with 50% ethanol and is added in to 10mL of 50% ethanol solution. Then this material is centrifuged at a speed of 4000rpm for 6 minutes to create one or more pellets. After that supernatant fluid is decanted and the pellet is suspended in freshly prepared Nathan Alcohol formalin substitute which contains 9 parts of 100% ethanol and 1% of 40% formalin. Finally, the pellet is taken out after about 45minutes of fixation and it is processed as routine histopathological specimen processing. [8]

2.5. Colloidon Bag Technique

Colloidon bags has to prepared by pouring the colloidon solution to the top of a polypropylene plastic or pyrex conical centrifuge tubes. The colloidon bags are filled with distilled water and they are stoppered with paraffin wax. Immediately before using the bag distilled water should be discarded and it should be added the fine needle aspiration which is suspended in 10% neutral buffered formalin. Then this is centrifuged at a speed of 2500rpm for 10 minutes. The supernatant is discarded and the sediment is placed in a tissue cassette and processed as routine histology specimens [53].

According to a study conducted at Tribhuvan University

Teaching Hospital, Kathmandu, there is no significant difference in the quality of the cell block prepared by plasma thromboplastin method and collodion bag techniques. Further both the techniques are cost effective [53]

3. Diagnostic Approaches with Cell Block Preparation

Preparation of cell blocks bridges the gap between cytopathological diagnosis and the histopathological diagnosis. Studies have found that less cellular dispersion and concentration of cellular material, preserved tissue architecture, availability of multiple sections for special staining, maximum utilization of the specimen and easy storage of cell blocks have aided in the yield of accurate test results in cell block studies over cytopathology smears. [9, 5]

The diagnostic accuracy, sensitivity and specificity of the test results generated from cell block techniques are greater than those of the cytopathological smears suggesting that the cell block technique is more effective than routine

cytopathological smears and this technique facilitates tumour classification. [10] Sensitivity of FNAC results are higher than the specificity of FNAC results. [11] (*Table 1*) It has been detected that 12.5% of inadequate specimens in direct smears could yield a diagnosis from cell blocks and the combined use of direct smear with the cell block increases the efficacy of the results up to 95.7%.-100%.(Cristo, 2016) Further, 68.4% of cases have given valuable additional information in cell block technique than direct smears [13]. Cell block preparation is a technique which can reduce the rate of unsatisfactory specimens. Cell block technique was capable to reduce the inadequate specimen percentage of 15% of FNAC smears to 5.8%. [14] Also it is proven that cell block studies are effective in decreasing background obscuring material and it has a better preservation of the architecture [15]. With the high level accuracy of the cell block technique results, incidences of repeat FNAC has reduced. [16] Therefore, it's considered that cell blocks should be prepared from every cytology specimen whenever possible to strengthen the results.

Table 1. Comparison of the sensitivity and specificity of FNAC smears and Cell blocks.

Study	Sample size	Sensitivity of FNAC smears	Specificity of FNAC smears	Sensitivity of cell block smears	Specificity of cell block smears
[9]	148	94.90%	93.75%	98.92%	96.30%
[5]	90	-	-	93.75%	93.75%
[11]	89	77%	100%		
[37]	358	93%	89%	100%	90%

Though FNAC smear preparation is safe, reliable and minimally invasive to diagnose the pathological conditions of the thyroid gland, there are reported cases in which results give false positive and false negative diagnosis when compared to the gold standard histopathological diagnosis [9, 13]. In a study by Ahmed, Medhi and Das, in 2015 where ten specimens out of the ninety specimens used for FNAC smear diagnosis were identified as follicular adenoma instead of colloid goiter. Further in the same study one case of medullary carcinoma was diagnosed as a follicular neoplasm by FNAC results. According to Thanigaimani and Murali, 2016, one case of follicular variant papillary carcinoma was diagnosed as follicular neoplasm with atypia in cytology smears.

Traditional cell block smears were used as an adjuvant to cytologic smears. [18] However, as more than one smear with the same cellularity can be prepared from a single cell block, the smears can be subjected to more advanced ancillary techniques like immunocytochemistry, molecular diagnosis, special staining and ultrastructural observation which permits to increase the diagnostic accuracy of the test results [7]. It is very important to identify the type and the grade of the thyroid nodule as tumor prognosis and the treatment plan can only be predicted with the aid of laboratory findings.

Immunocytochemistry is a technique which uses the antigen antibody reactions to identify specific types of receptors present on the cell membrane, cytoplasm or the nuclear membrane. It has been resolved that immunocytochemistry is an excellent method for the

differential diagnosis of thyroid lesions, when utilizing immunocytochemical markers such as CD56, CK-19, HBME-1, 34 β E12 and Galectin 3.

CD56 marker is usually present on follicular epithelial cells of normal thyroid tissue and it visualizes as a diffuse staining on the membrane. CD56 can be used to differentiate papillary thyroid carcinoma from follicular thyroid carcinoma as this marker is absent or low in papillary thyroid carcinoma. [19] Thus it can be used as a marker to categorize tumors according to the type of thyroid carcinoma. CD56 marker is also present in benign tumours. However other markers like HBME-1 or 34 β E-12 markers are negative in benign tumours and enable proper categorization of tumours. [20] HBME-1 is an antigen on mesothelial cell surfaces. This HBME-1 antigen is reactive for both papillary and follicular thyroid carcinomas in tissue sections as well as fine needle aspiration biopsy but, papillary carcinoma shows a higher reactivity when compared to follicular carcinoma. [21, 22] Therefore, this marker can be used to differentiate benign tumours from malignant tumours. Cytokeratin 19 (CK-19) is a low molecular weight cytokeratin present on simple or glandular epithelia of normal and neoplastic areas. This marker shows a diffuse and strong staining pattern in Papillary Thyroid Carcinoma. [23] Galectin-3 is another immunohistochemical marker used in differential diagnosis of thyroid carcinoma. This marker is expressed in malignant tumours while not in benign follicular lesion [50]. Galectin-3 is a β -galactoside binding protein and it is involved in cell signaling. It can be present intracellularly in

the nucleus, cytoplasm and in cell membrane or it can be secreted into extracellular matrix. Galectin-3 can also involve in physiological processes like cell adhesion, cell activation and chemo attraction and involve in pathological conditions like cancer progression and tumour metastasis [24].

According to conducted studies (*Table 2*) to assess the diagnostic value of several immunohistochemical biomarkers in the differential diagnosis of thyroid tumours has detected that HBME-1, Cytokeratin 19 and High Molecular Weight Cytokeratin expression has a significant association with Differentiated Thyroid Carcinoma. Also use of combinations of markers have a greater sensitivity in differentiating carcinoma. CK-19 and HMBE-1 aids to differentiate papillary carcinoma and follicular carcinoma. Follicular variant papillary carcinoma express Ck-19 and HMWCK in a higher specificity than Follicular carcinoma and there is a higher expression of HMWCK in follicular carcinoma than follicular adenoma. In Differentiated Thyroid Carcinoma HBME-1 expression is 91.3%, CK-19 expression is 90.3%, HMWCK

expression is 52.4%. In Papillary carcinoma 91.7%, 96.7% and 59.7% expressions are observed with HBME-1, CK-19 and HMWCK respectively. In follicular carcinoma HBME-1, CK19 and HMWCK expression is 88%, 44% and 0% respectively while 31.5%, 16.9% and 0% expression in benign thyroid nodules. [20] Absence of CD56 immunohistochemistry biomarker in Papillary Thyroid Carcinoma helps to differentiate it from benign thyroid tumours. Also CD56 marker is 98.6% sensitive and 95.8% specific in it's expression in Papillary Thyroid Carcinoma than other follicular lesions. [19] The same study has found that most sensitive and specific immunohistochemical biomarkers are CD56 negativity, HBME-1 positivity and Galectin-3 positivity. [25, 26]. Therefore, CD56 evaluation in fine needle aspirates is a good marker to rule out papillary thyroid carcinoma as the sensitivity is 96%. But, specificity is 69%. Further, combination of CD56 with HBME-1 increase the diagnostic accuracy of results. [27]

Table 2. Use of immunocytochemical markers for the diagnosis of thyroid nodules.

Research	Immunocytochemical markers used	Diagnosis
[38]	HBME-1, CK-19, 34βE12, Galectin 3, CD-15, CA 19-9	Papillary carcinoma positive for all the markers. 34βE12 is the most suitable marker.
[39]	CK-19	Useful tool in the diagnosis of papillary thyroid carcinoma
[36]	CK-19, HBME1	ICC is recommended as an effective diagnostic aid and therapeutic follow up of thyroid nodules.
[40]	CK-19, HBME-1, Galectin 3	Combined use of markers increases the accuracy of the results
[41]	CK-19, Galectin 3, HBME-1, CD-44, E cadherin, CD-56	Positive immunoeexpression of CK-19, Galectin 3, HBME-1 and CD-44 increases the diagnostic accuracy of papillary thyroid carcinoma while the loss of E cadherin and CD-56 express as a feature of malignancy
[36]	CK-19, HBME-1	Can reduce false positive and false negative results of singal morphological analyses.
[42]	B-catenin	100% sensitive in cribriform morula variant of papillary thyroid carcinoma
[43]	HBME-1, CD15	HBME-1 is a sensitive marker for both usual type of papillary carcinoma and follicular variant papillary thyroid carcinoma, Sensitivity is low and specificity is high in CD15
[44]	CD79a, CD 03, CD 30, cytkeratin	FNAC in combination with immunocytochemistry increases the diagnostic efficacy of thyroid lymphoma.
[45]	CD 147	CD147 expression correlates significantly with the degree of dedifferentiation of thyroid carcinoma CD-56 has a low specificity with a high sensitivity. Combination of CD56 with HBME-1 increases the specificity. Therefore, CD56 can be used as a preoperative marker for the diagnosis of Bethesda category 111 tumors.
[46]	CD56, HBME-1	
[47]	Cytokeratin 19, galectin 3, HBME-1,	CK-19 showed best results in the Bethesda category 02 cases and CK19 and HBME-1 are better markers for Bethesda 5, 6 tumors

Most of other carcinomas, and thyroid carcinoma results due to the accumulation of multiple genetic and epigenetic alterations in the genome. These can be due to point mutations or due to chromosomal rearrangements. Common sites where point mutations occur are BRAF gene and RAS gene while RET/PTC and PAX8-PPARγ result due to chromosomal rearrangements. Activation of MAPK and P13K-AKT signaling pathways by their mutations leads to tumorigenesis and thus leading to carcinoma. BRAF gene mutation is the most common mutation in thyroid cancers including Papillary Thyroid Carcinoma [28]. This mutation results due to replacement of a glutamine amino acid by valine amino acid at 1799th position of this gene, small frame deletions, insertions or chromosomal rearrangements. [29] BRAF gene mutation causes the production of BRAF-V600E mutant protein which activates the BRAF kinase and then MAPK signalling pathway. This mutation occurs in 45% of Papillary Thyroid Carcinoma [30, 31]. TERT gene, the catalytic sub unit of the

gene is responsible to keep the cells immotile by maintaining the telomere length at the end of chromosomes. Two mutations can occur in this TERT promoter region namely C228T and C250T leading to shortening of telomere length and thus increase the rate of cell replication and carcinogenesis. C250T is gene mutation is rare. However, both C228T and C250T are mutually exclusive. This gene has been identified as the best marker of aggressiveness, poor prognosis and distance metastasis of all the types of thyroid carcinoma. [31-35]

4. Conclusion

Though the thyroid nodules are relatively common in 7% of the population, their malignancy rate is low (5-10% of cases) [36]. However, the patients are subjected to unnecessary surgeries due to false positive diagnosis of the benign lesions

during the pre-operative period. Further, there are reported cases where re-operations done on the patient due to false negative diagnosis made in the pre-operative period. As tumor prognosis can be predicted by the expeditious and accurate diagnosis during the preoperative period it is essential to make better diagnostic strategies during the preoperative period. In a resource poor set up a convenient diagnostic method is the assessment of fine needle aspiration smears as it is a minimally invasive, cost effective and less time consuming method. However, poor cellularity and loss of tissue architecture in fine needle aspiration smears leads to false negative diagnosis and make obstacles in the differential diagnosis of thyroid tumors. Cell block technique is an effective alternative to fine needle aspiration smears which can overcome most of the defective features in FNAC smears. Further, as more than one smear with the same cellularity can be prepared from one cell block, it can be subjected to more advanced diagnostic methods like immunohistochemistry and molecular diagnosis which increases the diagnostic efficacy of thyroid tumors. Finally, the expeditious and accurate diagnosis of thyroid nodules during the preoperative period will prevent unnecessary total or partial thyroidectomies or re-operative procedures. The cell block preparation for thyroid fine needle aspiration samples improves the pre-operative diagnostic accuracy of suspicious thyroid nodules in a resource poor setup.

Conflicts of Interest

Authors declare that there are no conflicts of interest.

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