



## A Study on Diagnostic Utility of Cell Block Preparation of Fine Needle Aspiration Material

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### Abstract

#### INTRODUCTION:

Fine needle aspiration cytology (FNAC) is a reliable and simple procedure for all type of lesions. However, false negatives can occur with the FNAC procedure alone. Hence, cell block preparation from the aspirated material can be used to provide further information and the present study was conducted to compare the conventional FNAC smear morphology with cell block histopathological sections.

#### MATERIALS AND METHODS:

This was a study of 200 specimens from patients performed in the cytology section of pathology department. The remaining material in the needle hub and syringe after making the FNAC smears was rinsed with saline and utilized for cell block preparation after treatment with plasma thromboplastin and stained with hematoxylin and eosin.

#### RESULTS:

In our study, we analyzed the most common site and it was thyroid being commonest -100 (50%). Among these 200 cases, cytology smears were adequate in 183 cases and cell blocks showed adequacy in 185 cases. Among which both FNAC and cell block cases which were adequate 177 cases. Based on this the following results were obtained for cell block, Sensitivity: 95.6%, Specificity: 60%, PPV: 96.7%, NPV: 52.9%, Accuracy 93% and Kappa statistics: 0.524

#### CONCLUSION:

Cell block preparation increases cellular yield of FNA by capturing any small tissue fragments in bloody aspirates. Cell block preserves architectural pattern of tissues and cell morphology in sections. Although cell block preparation from fine needle aspirates is time consuming and increased cost, it is balanced by its utility and its ability to increase adequacy of cellularity and sensitivity of diagnosis.

**Keywords:** Cell block, Fine needle aspiration.

### INTRODUCTION

Fine needle aspiration has become an increasingly common procedure which eliminates costly diagnostic procedures. It provides safe diagnosis. At times FNAC do not provide proper information for a precise diagnosis and hence the risk of false negatives is always present.<sup>1</sup>A successful FNAC requires an experience aspirator, adequate specimen and

cytopathologist as well as high quality specimen preparation. In spite of this, around 20% of FNAC specimens are classified as non - diagnostic due to poor specimen preparation and scant cellularity. After preparing cytology smears, there is some residual material left behind which contains valuable diagnostic evidence and tissue fragments in needle hub

and syringes which cannot be processed by routine cytology technique. Therefore, additional information can be obtained via the preparation of cell block from the residual material of FNA which ensures maximum utilisation of available material.

A cell block is a condensed group of cells which is created from a fine needle aspirate that is fixed and paraffin embedded. The main advantage of cell block is to make multiple sections. Thus, cell block helps to perform ancillary studies such as in situ hybridisation, histochemical and immune histochemical stains.<sup>1</sup> Cell block ensures optimal preservation of histochemical and immunocytochemical properties. In general, the staining results obtained in the cell block sections agreed with those of the surgical specimens. Another advantage is the possibility of storing cell block for retrospective studies, especially when research is necessary<sup>2</sup>.

Although cell block is one of the oldest techniques, with the advent of newer techniques like fluorescence techniques, cytocentrifuge, preparation with membrane filters like Millipore and nucleopore, flow cytometry, molecular techniques and electron microscopy, cell block was abandoned by many laboratories. In view of the added advantages of cell block, it should be utilized routinely in cytology in order to increase the diagnostic efficacy of cytodiagnosis. It will avert the need for repeat FNA for non-diagnostic cases. If smears and cell block get concordant impression, then the need for biopsy can be obtained<sup>3</sup>.

Based on above understanding of cell block's objective of our study is to assess the morphology of various fine needle aspiration and cell block specimens and to establish morphologic standards. Also, to compare the conventional FNAC smear with cellblock sections and to evaluate the usefulness of cell block as an adjunct to smears in establishing definite diagnosis. Furthermore, to study advantages and disadvantages of cell block techniques over conventional FNAC smears and to establish the cost effectiveness of cell block technique.

## MATERIAL AND METHODS

The present study was done as a prospective cross-sectional study carried in a tertiary care teaching hospital for a period of two years. A total of 200 patients coming for routine investigation of FNAC to

the cytology section, Department of Pathology referred from various hospital departments and inpatients from various wards were taken for the study. All age group patients were included in the study.

After explaining about the procedure to the patients in local language, complete clinical history was obtained. Local examination was done. All the details were entered in the proforma. After explaining about the procedure, patients were taken for FNAC. FNAs near vital structures and deep-seated lesions were done in Radiology department as USG guided FNAC under the guidance of radiology faculty.

## Technique of cell block preparation:

In this study, with little modification, technique described by Koss<sup>4</sup> in the year 1991 was employed. Normal saline (Fredrick Mayhall et al)<sup>5</sup> in place of 10% alcohol formalin was used to flush the needle and syringe as clotting action of plasma and thromboplastin is inhibited by alcohol. After preparation of FNA smears, the remaining material in the needle hub and syringes was utilised for preparation of cell block. The residual material in the needle hub and syringes was obtained by flushing with normal saline. Paraffin blocks were made and paraffin cell blocks were cut to 4 – 5 microns thickness like routine paraffin blocks. They were mounted on glass slides according to standard histopathologic techniques. The sections were stained with Hematoxylin and Eosin. The reporting of various organ smears and final diagnosis were given as per Koss et al<sup>4</sup> and Orell et al<sup>6</sup>.

Cytology report was given in accordance with cellularity, cytomorphology, background and diagnosis. Cell block report was given based on cellularity, tissue architecture, background, and diagnosis. The diagnosis of cytology smears was correlated with cell block. The data was entered and analyzed with SPSS software version 22. Descriptive analysis was done, sensitivity, specificity, Kappa statistics were calculated and p value was used to find the association.

## RESULTS:

The present was done with a sample size of 200. The material comprised of FNA from different sites and smears were prepared from them. The residual material in needle bulb and syringes were processed using plasma thromboplastin as cell block. FNAC

smears and cell blocks were individually studied and their results were compared. Out of 200 cases, the maximum number of cases 126 (63%) cases were in the age group of 31 – 64 years of age, followed by 55 (27.5%) in 19-30 years age group and rest in other age group. Out of 200 cases, females 146 (73%) were more common with male female ratio of 1:2.7.

In our study, we analyzed the most common site of FNAC done and it was thyroid being commonest -100 (50%), followed by lymph node (N=44), Breast (n=40), soft tissue (n=10) and salivary gland (n=6). Among these 200 cases, cytology smears were adequate in 183 (91.5%) cases and cell blocks showed adequacy in 185 (92.5%) cases. FNAC smears were inadequate in 17 (8.5%) cases while cell blocks were

inadequate in 15 (7.5%) cases. Among which both FNAC and cell block cases which were adequate 177 (88.5%) cases, whereas only FNAC adequate in 6 cases, only cell blocks adequate in 8 cases and both were inadequate in rest of the cases. Out of 177 cases, 173 (97.7%) cases diagnosis of FNAC and cell blocks were correlated. 4 (2.3%) cases diagnosis of FNAC and cell blocks were non- correlated.

Out of 200 cases, 182 cases have correlation of diagnosis between FNAC and cell block and 18 cases have non-correlated diagnosis. p value <0.05 significant (0.0038). There is 100% correlation of diagnosis between FNAC smears and cell blocks in lymph node and soft tissue region.

**TABLE NO 2: Distribution of cases with correlated diagnosis between FNAC and Cell Blocks: (n=177)**

S.No	Site of FNA	Total adequate cases	Number of correlated cases	Percentage of adequate individual cases	Number of non-correlated cases	Percentage of individual organ cases
1.	Thyroid	92	90	97.8	2	2.2
2.	Breast	32	31	96.9	1	3.2
3.	Lymph node	40	40	100	0	0
4.	Soft tissue	9	9	100	0	0
5.	Salivary gland	4	3	75	1	33.3
	Total	177	173	97.7	4	2.3

There were 4(2.3%) cases with non- correlated diagnosis between FNAC and Cell blocks. Out of 90 cases from thyroid, 2 (2.2%) cases showed non- correlation of diagnosis. One case which showed suspicious of malignancy in FNAC smears showed malignancy in Cell block and the other showed Papillary carcinoma of thyroid in Cell block. One case in FNAC showed suspicious of malignancy in salivary gland and showed infectious in Cell block.

**Table 2: Cases with inadequate FNAC and adequate cell blocks according to site:**

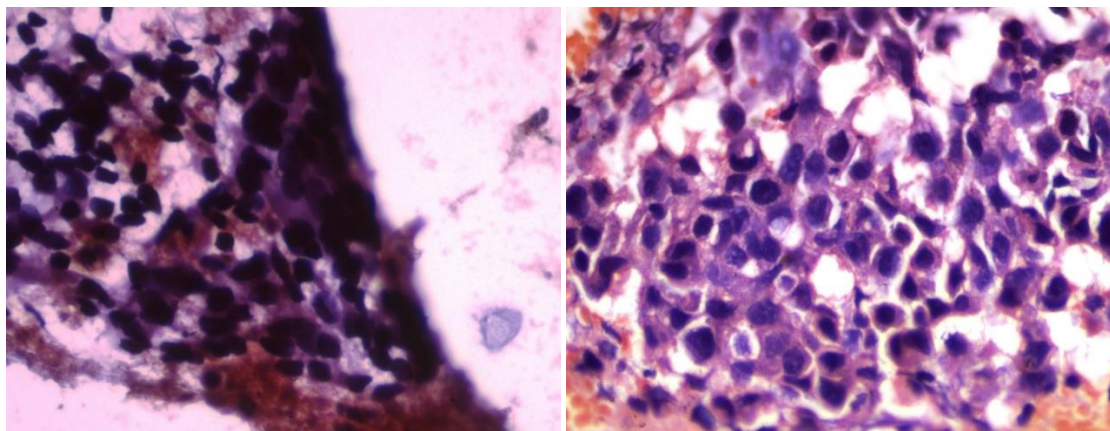
S.No	Site of FNA	Number of inadequate FNAC cases	Total number of cases	Percentage of individual organ cases
1.	Thyroid	6	100	6
2.	Breast	0	40	0
3.	Lymph node	0	44	0
4.	Soft tissue	0	10	0
5.	Salivary gland	2	6	33.3
	total	8	200	4

Out of 200 cases, 6 cases of thyroid, 2 cases of salivary gland were inadequate on FNAC smear and adequate on cell blocks. Out of 200 cases, 6 cases were adequate on FNAC smears and inadequate on cell blocks.

**TABLE 3: Cases with adequate FNAC and inadequate cell blocks according to site:**

S.No	Site of FNA	Number of inadequate on cell block	Total number of cases	Percentage of individual organ cases
1.	Thyroid	0	100	0
2.	Breast	4	40	10
3.	Lymph node	1	44	2.3
4.	Soft tissue	1	10	10
5.	Salivary gland	0	6	0
	Total	6	200	3

When cell block was used as an adjunct to FNAC, percentage of adequacy increased as compared to FNAC alone. Maximum increase in percentage was observed in lymph node. In lymph node the percentage of adequacy was 93.1% with FNAC alone whereas when cell block was used as an adjunct it increased to 97.7%.



**Figure 1: Malignancy in breast - FNAC smear and cell block**

The overall adequacy of FNAC smears and cell block were 91.5% and 92.5% respectively. Combined adequacy of FNAC and cell blocks were 92%. Hence based on this the following results were obtained for cell block, Sensitivity: 95.6%, Specificity: 60%, Positive predictive value: 96.7%, Negative predictive value: 52.9%, Diagnostic Accuracy (Index of validity & efficiency): 93% and Kappa statistics: 0.524 (Moderate agreement between the two diagnostic procedures).

## DISCUSSION

In the present study of 200 patients age group ranged from < 18 years to > 64 years and maximum number of cases were between 31 – 64 years of age with Male:

Female ratio was 1: 2. In a study by Shivakumarswami et al<sup>7</sup> Male: Female ratio was 1.4:1. The difference may be due to random selection of cases.

In the present study out of 100 aspirates from thyroid, 85 aspirates were in female and 15 from males. The female preponderance in the present study was in concordance with a study done by Sanchez N et al<sup>8</sup> where out of 80 cases from thyroid, 60 were from female patients.

In the present study, the most common site of aspirate was from thyroid, in Kern & Haber<sup>9</sup>, commonest site was breast, and in Leung & Bedard<sup>10</sup>, the most common site was lung while in Nathan et al<sup>11</sup> most common site was head, neck and face regions. The



sites were not correlated in the above-mentioned studies which could be due to random selection of cases.

In the present study correlated diagnosis of FNAC and cell blocks was 97.7%. Thus, the present study was consistent with the findings of Wojcik & Selvaggi<sup>12</sup>. Though diagnosis on FNAC smears and cell blocks were same in correlated cases, cell block showed better architectural pattern, which confirms FNAC diagnosis. In papillary carcinoma of thyroid, papillary pattern was better appreciated on cell block. According to Kern & Haber<sup>9</sup>, papillary carcinoma of thyroid can be reliably diagnosed on cell blocks by cell pattern.

Cell block showed better architectural pattern and cellularity (papillary pattern in papillary carcinoma of thyroid and granulomas were well appreciated in granulomatous lymphadenitis). Yamamoto et al<sup>13</sup>, emphasized that tumor morphology which could not be appreciated on cytology, could be better appreciated on cell blocks. De Bore et al<sup>14</sup> and Nathan et al<sup>11</sup> in their study showed that tissue architecture may be better appreciated on cell blocks.

In 17 cases FNAC smears were inadequate, while cell blocks were adequate. Thyroid was the most common site followed by lymph node. Out of 17 cases, many cases showed only blood on FNAC while cellblock showed diagnostic material. This can be due to entrapment of tissue fragments in blood clot which was processed further for cell blocks.

Orell et al<sup>6</sup> observed that samples when heavily admixed with blood, may show only blood on smears and few distorted cells trapped in blood clots. However, surprisingly in the cell block sections good tissue fragments may be found.

Out of 17 cases, one was from lymph node, one from breast and fifteen from thyroid, among which four showed malignancies on cell block. In 1984, Rofagaet al<sup>15</sup> found that in samples with aspiration of thyroid and recurrent carcinomas cell blocks were particularly very useful. In the present study, 3% cases were found to be adequate on FNAC and were inadequate on cell blocks. Maximum number of cases were from breast.

In the present study, 4.5% around 9 cases were inadequate in both FNAC smears and cell block. Out of these 9 cases 4 were from breast, 3 from lymph node and 2 were from thyroid. In vascular organs like

thyroid, even few passes for obtaining cellularity may lead to hemorrhagic aspirate. When cell block was used as an adjunct to FNAC there was increase in percentage of adequacy (92%) compared to FNAC alone (91.5%). According to the study done by Nathan et al<sup>11</sup>, cell blocks are adjunct to FNAC for making a more definite cytopathologic diagnosis. Adequacy of cell blocks in the present study was comparable with Basnet et al<sup>16</sup>.

Adequacy of FNAC was lower than cell blocks in the present study which is consistent with the study by Basnet et al (2012)<sup>16</sup>. Kuo et al<sup>17</sup> concluded in their study that combined analysis of FNAC and Cell blocks had more diagnostic adequacy than examining FNAC smears alone.

In the present study the cell blocks were described by Koss<sup>4</sup> using plasma thromboplastin. It showed better morphological details compared to simple cell block method. The problems with this method were that plasma thromboplastin had to be stored in refrigerator and brought to room temperature before use and it also increases the cost of cell block. The observed advantages in the present study were: Preservation of tissue architecture. Less cellular dispersion which helps in microscopic examination., By using the remaining material in needle hub and syringes maximum utilization of all available material is ensured., Fine needle aspirates can be stored as cell blocks. Multiple sections can be taken for special stains whenever needed.

This was similar to below studies, in 1982 Karnauchow & Bonin<sup>18</sup> observed a thick tissue particle that cannot be examined properly on FNAC smears, provided adequate material for cell blocks. Tatsula et al<sup>19</sup>, observed that cell Blocks were helpful when many immunocytochemical investigations were needed.

The disadvantages of the technique observed in the present study were: Cellular details were less clear in cell blocks as compared to FNAC smears., As compared to smears cost of cell blocks were high., Delay in diagnosis due to increased processing time. Similarly, Zito et al (1995)<sup>20</sup> observed the delay in diagnosis in his study. According to their study, though cell blocks increase the diagnostic accuracy of cytological smears, its routine use is impractical due to delay in their diagnosis. Hence, when multiple

sections are needed for immunohistochemical staining, cell block technique is valuable.

The observations of the present study are in agreement with other authors Basnet et al<sup>16</sup> and Nathan et al (2000)<sup>11</sup>. The importance of cell block has been stressed in all these studies. It not only increases the diagnostic yield but also provided an appropriate diagnosis and hence proper management. According to Chiu et al<sup>21</sup>, cell block elevates the diagnosis of FNA specimen to histopathological level. According to Yang et al<sup>22</sup>, cell block preparation is highly valuable in cytopathology as it provides histopathologic correlation and also multiple sections for immunohistochemical study.

### Conclusion

Cell block preparation is simple and reproducible and it uses routine safe laboratory chemicals for processing. Routine fine needle aspiration for cytological examination leaves behind some residual material which may contain valuable diagnostic material that can be captured by cell block preparation without repeating the FNA or any additional procedure. Cell block preparation increases cellular yield of FNA by capturing any small tissue fragments in bloody aspirates, with less dispersion. Cell block preserves architectural pattern of tissues and cell morphology in sections, though not as good as in smears, is familiar to histopathologist. It also allows examination of same cell from another angle. Immunohistochemistry and special stains can be performed on cell block which helps to subtype certain tumors and metastasis. By this, open biopsies can be avoided. Although cell block preparation from fine needle aspirates is time consuming and increased cost, it is balanced by its utility and its ability to increase adequacy of cellularity and sensitivity of diagnosis.

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