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Evaluation of Utility of Cytospin Technique in Fine Needle Aspirations

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Abstract

Fine needle aspiration cytology (FNAC) is an important diagnostic tool used preoperatively for diagnosis of palpable and non palpable mass lesions. Various techniques have been described for doing the procedure. *Aims & Objetive*: We conducted a study with the objective of evaluating role of cytospin technique in inadequate and hemmorhagic samples in cytology. To perform immunocytochemistry on the cytospin slides if required during the diagnosis. *Results*: 19 thyroid cases, 6 salivary gland cases, 30 lymph node cases, 12 soft tissue cases, 9 breast cases and 4 miscellaneous cases were done. Cellularity was inadequate in 1.3% cases in conventional FNAC whereas inadequacy was seen in 8.8% cases of cytospin slides. The cellular details were clear and satisfactory in 100% of the cases in both conventional and cytospin slides. The cytospin slides prepared from the residual hub material was diagnosed as colloid goiter whereas only abundant colloid was seen on conventional smears where opinion was not possible. Granulomas were missed with cytospin technique due to significant decrease in the background caseous necrosis and scattered cellularity. Immunocytochemistry on cytospin slides was convenient due to monolayered cells aiding in appropriate diagnosis. *Conclusion*: Cytospin can be used as an adjunct to routine smears where limited material or hemorrhagic material is present. Special ancillary tests like immunomarkers and special stains can be done on these slides which aids in rapid and definitive diagnosis.

Keywords: Conventional FNAC; Cytospin Technique; Immunochemistry.

Introduction

Fine needle aspiration cytology (FNAC) is an important investigative tool done routinely for diagnosis of mass lesions. It is minimally invasive and helps us in identifying benign and malignant lesions. It aids in planning apt management for the patients. But FNAC does have certain limitations when the sample aspirate is inadequate or hemorrhagic resulting in no opinions [1]. Cytospin is a technique in which samples are centrifuged at high velocity for a specified time which enables adhesion of the cells to the glass slide in a specified area of one centimetre. As the area to be analysed is small and even if few cells are present in the aspirate it is localized to a definitive area enabling better analysis of the same. Many studies have been conducted comparing smears prepared from cytospin and direct smearing technique following FNAC with results both supporting and disapproving the routine utilization of cytospin technique [2,3].

We conducted a study with the objective of evaluating role of cytospin technique in inadequate and hemmorhagic samples in cytology. To perform immunocytochemistry on the cytospin slides if required during the diagnosis.

Materials and Methods

A prospective study was undertaken in BMCRI from January to December 2016. It included randomly

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selected 80 cases of FNAC done on outpatient basis. 5 ml syringes of 22 G were used to aspirate with 1-3 passes for adequate material. Four slides were made by expressing the material on the glass slide with 2 slides for wet preparation and 2 slides for dry stains. These routine conventionly prepared slidesfor wet preparation were kept in methanol for fixation and stained with H &E. Two slides which were air dried was stained with Giemsa stain.

The residual material in the hub was flushed with fixative containing methanol and 3% acetic acid in the ratio of 9:1. The material was then added to test tube and centrifuged at speed of 3000 rpm for 30 minutes. The ssupernayent is discarded. Same fixative is added again to the residual material and the cycle is repeated. Following this the residual material is added to cytospin instrument and Cytospin slides were prepared after centrifugation of the material in CYTOTEK machine for 30 minutes at 3000 rpm. The slides were stained afterwards by haematoxylin and eosin stain.

Both the slides were reported separately in a blinded fashion by the same pathologist and the diagnostic utility of cytospin slides were evaluated.

Results

The study included total of 80 cases which included 19 thyroid cases, 6 salivary gland cases, 30 lymph node cases, 12 soft tissue cases, 9 breast cases and 4 miscellaneous cases over a wide age range. Staining was satisfactory in all the cases. Cellularity was inadequate in 1.3% cases in conventional FNAC whereas inadequacy was seen in 8.8% cases of cytospin slides. The cellular details were clear and satisfactory in 100% of the cases in both conventional and cytospin slides.

Out of 19 thyroid cases 11 cases were diagnosed as nodular goitre and 8 cases were diagnosed as Hashimoto's thyroiditis on cytospin. On conventionally prepared slides 10 cases were diagnosed as nodular goiter, 8 hashimoto's thyroididtis. In one case there was only abundant colloid and no opinoin was possible on conventional FNAC slide. The cytospin slides prepared from the residual hub material in this case showed benign thyroid follicular cells in clusters with anisokaryosis and was diagnosed as colloid goiter.

Among 6 cases of salivary gland slides from both conventional FNAC and cytospin preparations concluded 2 cases as pleomorphic adenoma, 3 cases as chronic sialadenitis and 1 case as benign cystic lesion.

Majority of the cases were of lymph node where both the preparations reported 11 cases of reactive lymphadenitis, 7 cases as metastatic deposits of squamous cell carcinoma and 8 cases of suppurative lesion. 3 cases were diagnosed as caseating granulomatous lymphadenitis on conventional FNA slides. In these cases no conclusion was possible on cytospin slides as the caseating background which is a crucial diagnostic factor was not visualised. Even the granulomas were missed on cytospin slides due to low cellularity of cytospin slides. This resulted in 10% of cases being missed on cytospin preparation. One of the cases diagnosed as poorly differentiated carcinoma deposits in the lymph node on both conventional and cytospin slides, cytospin had an added advantage where immunocytochemistry(ICC) was performed with cytokeratin and diagnosis of squamous cell carcinoma metastasis was possible.

Similarly, in breast cases out of total 9 cases 4 cases (44%) of fibrocystic disease were diagnosed on conventional FNAC slides. As the residual material in needle hub after expressing the material for routine slides showed only sparse scattered epithelial cells no opinion was possible on cytospin slides. 3 cases of fibroadenoma and 2 cases of adenocarcinoma were diagnosed by both the preparations.

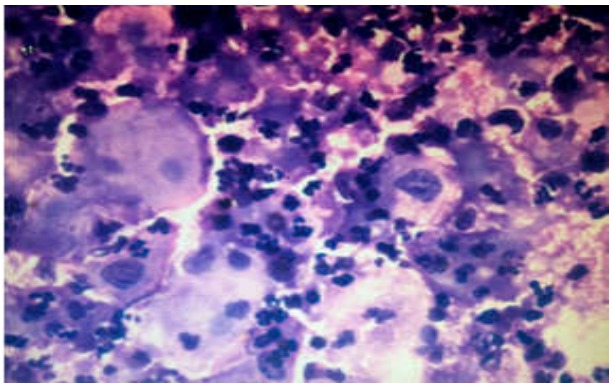

Fig. 1: Showing cytospin slide of well differentiated squamous cell carcinoma. The polygonal cells show hyperchromasia with irregular nuclear margins

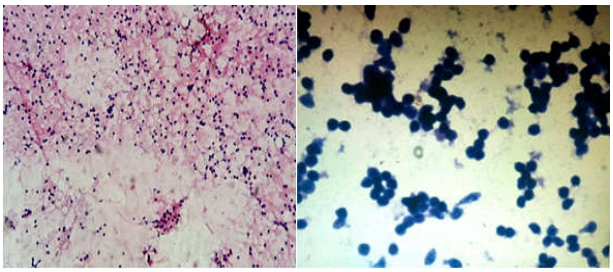

Fig. 2: Showing reactive lymphadenitis slides from both conventional FNAC and cytospin technique. **(A)** shows conventional slide with hemorrhagic background with polymorphous population of cells. **(B)** A clear background is seen on cytospin.

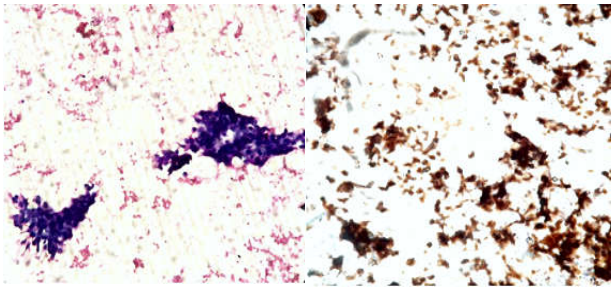

Fig. 3: Showing H& E slide and ICC slide in lymph node. **(A)** shows FNA slide with clusters of hyperchromatic cells showing nuclear overcrowding. **(B)** shows clusters and scattered cells with cytokeratin positivity.

Among soft tissue swellings 12 cases of lipomas were diagnosed by both cytospin and routine smears. Also 4 cases of epidermal inclusion cyst were picked up by both the preparations.

Discussion

Following FNAC the residual material present in the hub is usually discarded as it cannot be expressed on the slide but if utilised it provides very useful details for diagnosis. Various techniques have been utilised to retrieve the residual material which includes cytospin, thinprep and cell block technique. Ancilary techniques like cytospin with centrifugation technique and thinprep has helped in concentrating the residual material present in the hub onto the slides [4,5]. With this the chances to report a case tremendously increases, especially as the slide can be used for special tests like immunocytochemistry.

Cellularity of the smears was adequate in 98.75% on conventional FNAC were as adequacy was found in 91.2% cases in cytospin slides prepared from residual material. Studies have shown that slides from cytospin technique provide additional information supporting apt diagnosis in many cases. In a study by Liu et al, cytospins contributed additional information in 2% of cases beyond that obtained from routine smears [6]. In another study done by gupta et al, found that cytospin preparation yielded diagnostic material in 16% of cases where conventional smears were non-diagnostic [4]. However, in contrast the study by Stanley et al showed that needle rinse material in diagnosis of malignancy did not identify any additional malignancies [7].

Cellular details were satisfactory in all 80 cases. Methanol enables fixation and prevented air drying artefact. Acetic acid supported in haemorrhage free background thereby minimising artifacts as in Figure 1. Various solutions have been utilised by various authors like saline, 95% ethanol, 10% formaline and also commercially available cytofixatives.

In thyroid, cytospin provided additional cellularity in one case helping in diagnosis of colloid goiter. In other cases with both conventional and cytospin slides diagnosis was possible. Cytospin had an added advantage of non hemorrhagic background and concentration of the residual material in the hub. This reduces the repeat FNAs and thus will have better patient compliance. On the other hand a few disadvantages faced due to cytospin technique were background of the smears which aid in diagnosis such as colloid was markedly reduced. Adequacy of the material in hemorrhagic and scant aspirate could not be assessed immediately as in conventional smears. Various studies have also described changes in background material like significantly reduction in colloid and its appearance as droplets with fragmentation of large cell clusters emphasizing the importance of familiarising with cytomorphology of the cells in cytospin method [8,9]. Some authors have described similar results on both conventional and thinprep slides with preservation of cytomorphology of the cells in thinprep slides [10]. Ljung et al describes conventional smears to be better in the diagnosis of malignancy of thyroid due to better cellular preservation and background [11]. In our study good correlation was possible in slides from salivary gland prepared by both conventional and cytospin method. Faquin et al have suggested that cytospin technique alone should not be used in salivary gland lesions particularly those with background matrix and recommend its utility in cystic lesions [12].

The cytospin slides provided a monolayered sheet of cells with polymorphous population in a clean background and clear cellular details in cases of reactive lymphadenitis as in Figure 2. However, the background material like caseous necrosis was absent in cytospin slides. Also cells were more scattered and a well formed granulomas was not seen as in conventional smears. This resulted in no definitive diagnosis of tubercular lymphadenitis in cytospin slides. A high centrifugation speed and prior centrifuge of the samples probably resulted absence of caseous material and fragmentation of the cell clusters. Optimisation of the speed of centrifuge and its duration is probably necessary for different organs on cytospin. This technique can be used for immunocytochemistry with ease due to monolayered cells and small area of cell concentration as in Figure 3.

Conclusion

Cytospin can be used as an adjunct to routine smears where limited material or hemorrhagic material is present. Special ancillary tests like immunomarkers and special stains can be done on these slides which aids in rapid and definitive diagnosis. However factors like limited cellularity with altered morphological pattern should be taken into consideration and well understood prior to reporting.

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