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RESEARCH ARTICLE

IMPLEMENTATION OF CELL BLOCK TECHNIQUE ON FINE NEEDLE ASPIRATION FROM LIVER DISEASE

***Dr. Yasir Hakim**

Department of Basic Medical Science, College of Medicine, Dar Al Uloom University, Riyadh, Kingdom of Saudi Arabia

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***Corresponding author:* Dr. Yasir Hakim**

ABSTRACT

Objectives*:* The study was conducted in Dar Al Uloom University during the period (June - August 2017) to assess the implementation of cell block technique in the diagnosis of liver disease based on specimen adequacy and diagnostic accuracy, to evaluate the level of background staining, cellularity, nuclear, cytoplasmic preservation in cell block section, to compare the differences between the diagnostic results of cell block slides, conventional smears and to explore the feasibility of the use of cell block preparation in routine cytology. ***Methods:*** Eighty study subject were selected for the study, ultrasound guided fine needle aspiration was obtained from liver, then aspirate cells fixed in 40% formalin overnight, the supernatant fluid was decanted and settle cells warped in filter paper and then placed in a tissue cassette. All tissue cassette processed in an automatic tissue processor, the cell block were embedded in paraffin wax, and 4-6 micron were cut using standard rotary microtome. The sections were stained with Haematoxylin and Eosin (H&E) stain and cover with cover slip using DPX mounting media, and cytological smears was also stained with Haematoxylin and Eosin stain. ***Results:*** The result obtained that the high score (+3) in smear 41(51.3%) while in cell block 27(33.8%). Moreover when compared the final architecture preservation which include nuclear and cytoplasmic preservation, the nuclear preservation were the best by cytological smears than cell blocks, score (+3) in smears 43(53.8%) while in cell block 37(46.3%). In the comparison of cytoplasmic preservation between the two methods found that the cytological smears was the best, the high score (+3)42 (52.5%) while in cell block 35(43.8%) comparing to the background staining found that the cell block has mild background staining 55(68.8%) while smears 7(8.8%) ***Conclusion****:* In this study smear preparation was the best in comparison with cell block technique in preservation of cells, nucleus and cytoplasm of liver disease.

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INTRODUCTION

Cell block cytology is a technique used in cytopathology (in addition to smears) for evaluation of tissue from fine needle aspirations or fluid aspirations. cell blocks prepared from residual tissue fluids and fine-needle aspirations can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. It can be particularly useful for categorization of tumors that otherwise may not be possible from smears themselves (Zito *et al.,* 1995).The paraffin blocks that prepared from any suspension of cells or fluids are suitable for sectioning, staining, and microscopic study; cells are concentrated by centrifugation or filtering techniques, and the resulting aggregation is processed as if it were a solid specimen of tissue (Farlex Partner, 2012). In routine cytological practice, the cell morphologic changes in smears are not always obvious. Sometimes judgment is difficult; therefore the application of cell block may give a better presentation of detailed cytological architectural features. Also implementing histochemical or immunocytochemical staining has been a useful adjunct for establishing a more definitive cytopathological diagnosis. Furthermore, cell block is also a source of archival material for cytological research (Ceyhan *et al.,* 2006). Sometimes cytology does not provide sufficient information and the risk of false negative or undetermined diagnosis exist (kulkarni *et al.,* 2000). The cell block technique increases the chance to achieve a reliable result and also to help demonstrate better architectural patterns which could be of great help in the routine for approaching correct diagnosis (Mansy *et al.,* 2006). In spite of years of research progress, the diagnosis of many diseases, especially cancer, still requires light microscopic evaluation of a sample of cells. To make a diagnosis, pathologists look for alterations in cell structure, and for changes in the composition and organization of tissues (Fischer *et al.,* 2010). It is obviously an advantage to be able to make a diagnosis, or to guide therapy, based on the smallest possible biopsy sample; the smaller biopsy has fewer risks and complications for the patient (Fischer *et al.,* 2010). Cytology is the field that uses the smallest possible "micro biopsies" for diagnosis .The appeal of cytology is that it can provide a small but diagnostic sample. By minimizing risks and complications for detecting certain diseases, cytology can be used for the

screening of disease .When cellular level alterations alone are sufficient for a diagnosis, a minimal sample size is acceptable. For many diagnoses, however, it may be necessary to be able to recognize the larger-scale alterations in tissue architecture, or to study biochemical and molecular characteristics of the cells ; cell blocks fulfill this need (Fischer *et al.,* 2010). Cell blocks are micro biopsies embedded in paraffin. A standard histologic section, measuring four or five microns in thickness, shows the organization and cellular composition of a micro biopsy fragment. Generally, diagnoses can be made with the most confidence when combinations of cellular and tissue level morphology are present (Fischer *et al.,* 2010).

Immunohistochemistry (IHC) allows disease-specific antigens, or combinations of antigens, to be detected. Immunohisto chemistry can be performed on cytology preparations of micro biopsy samples, however there are many advantages in using cell blocks. IHC on cytology can trap antibodies or reagents in large tissue fragments, giving the impression of a positive staining reaction. Paraffin sections allow each part of a micro biopsy to have equal access to. Immunohistochemistry (IHC) reagents. Another advantage of using cell blocks for IHC is the relative ease of scoring or quantifying positivity on a per cell basis. Cell blocks are the ideal platform for. Immunohistochemistry (IHC) and molecular diagnostic ancillary studies. Immunohistochemistry (IHC) on cell blocks also allows the staining reaction to be correlated with larger-scale tissue architecture. For example, the finding of hepatocytes growing more than a few cells away from the CD31 positive endothelial cells within a tissue fragment is virtually diagnostic of hepatocellular carcinoma. Serial sections that can be cut from cell blocks allow multiple. Immunohistochemistry9 IHC0 reactions to be studied in the same sample. One tissue fragment 100 microns in diameter gauge fine needle) could be used to study 20. Immunohistochemistry (IHC) reactions in five micron serial sections, but it can only be studied in one immunohistochemical staining reaction in a cytology preparation. cell blocks are a convenient and stable means for archiving biopsies at room temperature, with many advantages over freezing, storing glass slides, or storage of liquid fixatives. Defining individually optimal therapies is becoming an essential duty for pathologists with the advent of new molecular-based treatments. Paraffin embedded tissue has emerged as the standard platform to achieve this goal of "personalized medicine'' (Fischer AH *et al.,* 2010).

METHODS

Ethical approval: The present study of implementation of cell block technique on fine needle aspiration from liver disease of certain patients groups were approved and performed in accordance with the regulations of college of medicine ,Dar U loom University Board, KSA and consent from the patients to guarantee confidentiality.

Study population and design: This was prospective and comparative study aiming to to assess the implementation of cell block technique in the diagnosis of liver disease based on specimen adequacy and diagnostic accuracy, to evaluate the level of background staining, cellularity, nuclear, cytoplasmic preservation in cell block section, to compare the differences between the diagnostic results of cell block slides, conventional smears and to explore the feasibility of the use of cell block preparation in routine cytology. Data were obtained from All liver disease patients attending University Teaching Hospital for ultrasound guide fine needle aspiration in the period between June to August 2017. Based on eighty liver ultrasound guide fine needle aspiration, each sample divided into two, one for cell block and the other for routine cytology smears, then compared together. Guided by Endoscopic ultrasound fine needle aspiration to obtain cellular material for cytological examination & Liver ultrasound guided fine needle aspiration cytology technique. Data was analyzed by program statistical package for social Sciences (SPSS).

RESULTS AND DISCUSSION

In the current study eighty samples of liver biopsies were used from different patients according to the ages and gender, based on the ages of the patients F -1 the highest age group was 61-70 years 19 (23.8%), in compare between females to males in (F-2) males were 45(56%) and females were 35(44%). And the comparison between routine cytology and cell block technique was done including cellularity, nuclear and cytoplasmic preservation and background staining. The high score (+3) in smear 41 (51.3%) while in cell block 27(33.8%), the moderate score (+2) in smear 30(37.5%) while in cell block 18 (22.5%), the low score (+1) in smear 9(11.3%) while in cell block 35(43.8%). Assessment of agreement of cellularity between the two method of sample preparation the following were obtained : P value of high score (+3) 0.000 (highly significant) because most of cell block samples loss their cells during preparation in cell block this agree with Kulkarni *et al.* 2000, reported that the main problem in cell block technique is risk of losing the cellular material during preparation it's important to mention that cell block component may be missed during sectioning due to low cellularity. Table.1.And in Table -2 in Comparison between smears and cell block in nuclear preservation. The high score (+3) in smear 43 (53.8%) while in cell block 37(46.3%), the moderate score (+2) in smear 33(41.3%) while in cell block 35(43.8%), the low score +1 in smear 4(5%) while in cell block 8(10%). P value of high score between two method of preparation were 0.003 - The nuclear and cytoplasmic preservation in smears better than cell block, this may be due to many steps in processing of cell blocks which affect in to the cell agree to Shehnaz Khan *et al*, 2012 reported that the agreement architectural preservation in FNA was 100%, compared to only 47% in cell block sample.

In Table 3: Tto comparison between smears and cell block in cytoplasmic preservation: The high score +3 in smear 42 (52.5%) while in cell block 35(43.8%), the moderate score +2 in smear 36(45%) while in cell block 34(42.5%), the low score +1 in smear 2(2.5%) while in cell block 11(13.8%). P value of high score between two method of preparation were 0.005 - The cytoplasmic preservation in smears better than cell block, this may be due to many steps in processing of cell blocks which affect into cells agree to Shehnaz Khan, *et al*. 2012 reported that the agreement architectural preservation in FNA was 100%, compared to only 47% in cell block sample. In Table 4: Comparison between smears and cell block in background staining: The sever background staining +3 in smear 42 (52.5%) while in cell block 10(12.5%), the moderate background staining +2 in smear 31(38.8%) while in cell block 15(18.8%), the mild background staining +1 in smear 7(8.8%) while in cell block 55(68.8%). P value of high score is between two method of preparation were 0.008 .cell block give less background staining than smears, this agree to the study of Raafat Awad Hegazy *et al.* 2014 said that evaluated the

Table 1. Comparison of cellularity between smears and cell block

Technique	Cellularity			Total
	1+ (0-10%)	2+ (11-50%)	3+ More than 50%	
Smear	9 (11.3%)	30 (37.5%)	41 (51.3%)	80 (100%)
Cell block	35 (43.8%)	18 (22.5%)	27 (33.8%)	80 (100%)
Total	44(27.5%)	48(30%)	68(42.5%)	160(100%)

Table 2. Comparison between smears and cell block in nuclear preservation

Technique	Nuclear Preservation			Total
	1+ (0-10%)	2+ (11-50%)	3+ More than 50 %	
Smear	4 (5%)	33 (41.3%)	43 (53.8%)	80 (100%)
cell block	8 (10%)	35 (43.8%)	37 (46.3%)	80 (100%)

Table 3. Comparison between smears and cell block in cytoplasmic preservation

Technique	Cytoplasmic preservation			Total
	1+	2+	3+	
Smear	2 (2.5%)	36 (45%)	42 (52.5%)	80 (100%)
cell block	11(13.8%)	34 (42.5%)	35 (43.8%)	80 (100%)

Table 4. Comparison between smears and cell block in background staining

Technique	Background			Total
	1+ Mild background staining	2+ Moderate background staining	3+ Sever background staining	
Smear	7 (8.8%)	31(38.8%)	42 (52.5%)	80 (100%)
cell block	55 (68.8%)	15 (18.8%)	10 (12.5%)	80 (100%)

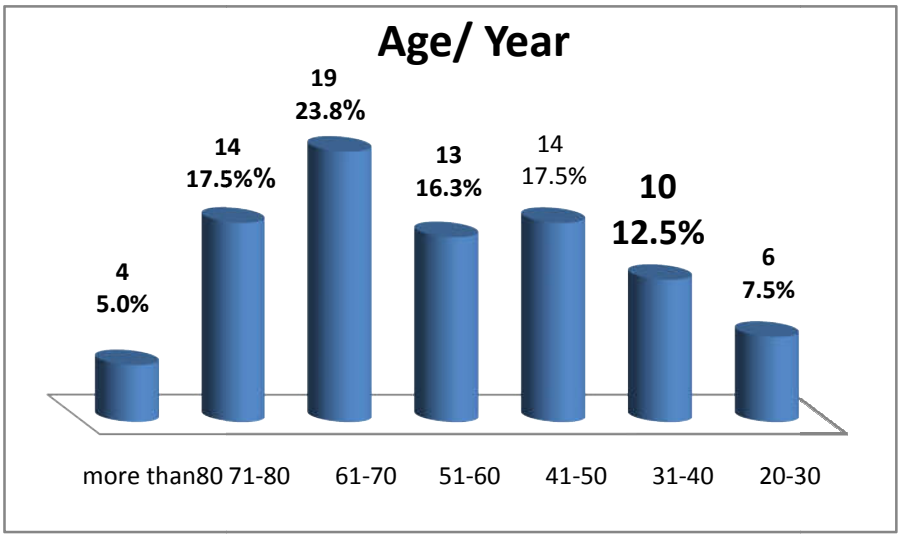

Fig. 1. Showed the age of the patients

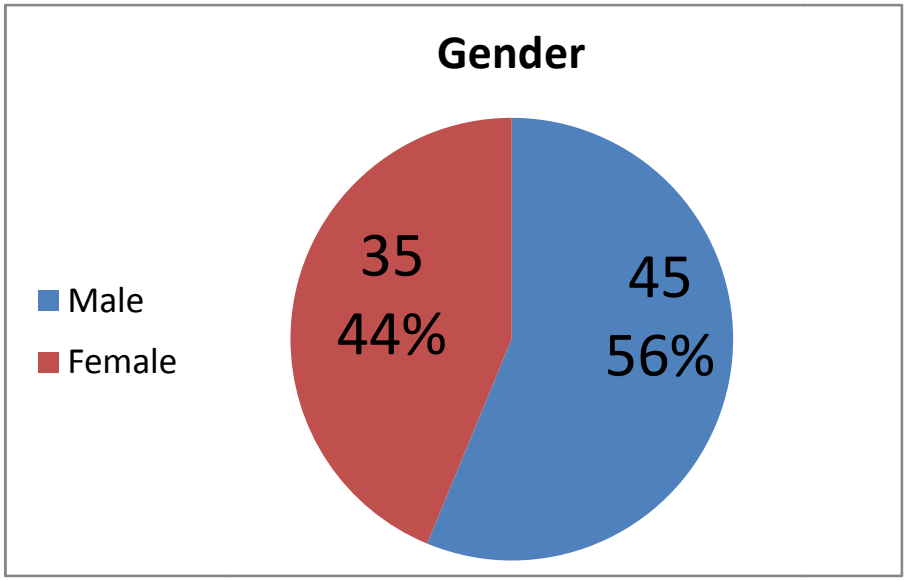

Fig. 2. Gender of the patient

importance of the combined use of fine needle aspiration cytology (FNAC) and cell block in the diagnosis of different breast lesions, in this study 310 cases (301 females and 9 males) with breast swelling coming to cytopathology unit, FNA and cell block were performed, the study showed that combining fine needle aspiration (FNA) with core biopsies has been increase diagnostic accuracy, this study suggests that combining a smear preparation of breast fine needle aspiration (FNA) with the cell block can also combine the advantages of both approaches, the sensitivity was 94%, specificity was 98%.

Conclusion

The study support that the smear preparation was the best in comparison to cell block technique in preservation of cells, nucleus and cytoplasm of liver biopsy specimens.

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