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Original Research Article

Role of Cell Block Technology as an Adjunct to Fine Needle Aspiration in Evaluating as well as Differentiating Liver Lesions

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Abstract

Aim: The aim of the study was to find the role of cell block technology as an alternative to biopsy in identifying liver lesions.

Material & Methods: A Retrospective study with 500 cases was done at department of Pathology, IGIMS, Patna, Bihar, India in between July 2015 to Jan 2017 . FNAC of the liver lesions were done and smears were prepared for routine staining (HE, PAP, and MGG), the rest of the material was submitted in 60% ethanol for cell block preparation. Usefulness of cell block preparation was evaluated, and the final diagnosis correlated with the biopsy results.

Results: There were 60% male and 40% females. Hepatocellular carcinoma was in the range of 46-82 years with a mean of 66.4 years while metastatic age range was 40-80 years with a mean of 58.2 years. On cell block, with or without immunohistochemistry, 75 cases (15%) were positive for hepato-cellular carcinoma, 380 cases (76%) were positive for metastatic lesions, 10 cases (2%) were suspicious of malignancy and 35 cases (7%) were designated as benign lesions. Morphology was observed from the smears obtained with MGG, PAP and H&E routinely from the cell block preparation. Special stain was PAS (to look for mucin) and reticulin (to look for trabecular strand) was also performed on cell block preparation. A detailed statistical analysis showed sensitivity of all the lesions diagnosed through cell block method to be 97.50% with positive predictive value of 98% and P-value highly significant at <0.00001. Diagnosing metastatic carcinoma was also very accurate with positive predictive value of 98.2%. Primary lesion like hepatocellular carcinoma with 100% positive predictive value, 92.8% sensitivity and significant P-value had very precise results on cell block. However, differentiating the various types of metastatic lesions on cell block was less on target with accuracy ranging from 66.66% to 100% for various carcinomas.

Conclusion: High precision of validity results of cell block technology in comparison with biopsy highlights its pivotal role in conjunction with supportive tests for diagnosing and differentiating liver lesions as well as identifying primary sites in liver metastasis.

Keywords: Cell block, Cytopathology, Diagnostic utility, Immunohistochemistry Cellblock, Hepatocellular carcinoma, Metastatic adenocarcinoma.

Introduction

Liver shows an exhaustive gamut of pathology, focal and diffuse; benign and malignant; primary and metastatic. The metabolic functions of the organ and its dual vascular supply make the management of liver neoplasms a challenge. Carcinoma of liver has a prevalence of 2-8% worldwide.[1] Most of the liver masses prototype can be suspected by the clinician with history, signs and symptoms, examination and correlation with radiological aids like USG, CT or MRI. However, confirmation needs a definitive pathological report, previously considered to be a histopathological report following a biopsy. Accurate localization and characterization are pertinent for management decisions as they delineate the neoplasms that are compatible with surgical methods from those that need only palliative therapy. Fine needle aspiration cytology (FNAC) plays an important role as it is rapid, less hazardous, and easy to perform diagnostic modality.[2] FNAC is quick, easy and helps the oncologist to plan out the management of patients. To differentiate between benign and malignant as well as primary and metastatic liver lesion is important Imaging techniques such as ultrasonography, CT/MRI scan can be used as a guide for FNAC of intra-abdominal lesions by adding to the yield of the aspirate and providing important diagnostic clues.[3] Diagnostic sensitivity of FNAC of liver varies from 67-100% and specificity 93-100%.[4] So FNAC has gained increased acceptance as surgical procedures are invasive and requires general anesthesia and hospitalization. However, FNAC sometimes does not yield information for precise diagnosis and there is always the risk of false negative/indeterminate diagnosis.[5,6] Here, an attempt to overcome the deficiencies of FNAC using cell block technology as an adjunct and compare that with a core needle biopsy. In these cases, cell block preparations can be helpful. Cell block is a cost-effective procedure and useful adjunct to smears to establish a more definitive diagnosis. It preserves architectural patterns like cell balls, papillae, and three-dimensional clusters with better nuclear and cytoplasmic preservation, intact cell membrane, crisp chromatin details.[7] Cell blocks can be used for histochemical stains, immunocytochemistry, and in insitu-hybridization. Blocks can be stored for the long term and multiple sections can be performed unlike cytological smears.[8] If properly done, it is very helpful especially using a small-bore tube and essentially converts cytology to histopathology, thus can be called Fine needle aspiration histopathology. Although FNAC with cell block may be costlier than a biopsy, it is logistically easier on the patients and has a much better compliance as sometimes biopsy has a negative psychological impact. Hence the aim of this study was to evaluate the scope and accuracy of cell block following FNAC with or without immunohistochemistry along with ancillary studies for diagnosing various liver lesions (especially SOL, space occupying lesions). Also, we aimed at evaluating the role of cell block for differentiating primary hepatic malignancy from metastatic lesions of the liver along with the use of cell block as an adjunct to FNA in sub typing the various metastatic carcinomas and identifying the source or the origin of the malignancy.

Material & Methods

This was a retrospective descriptive study carried out with 500 cases of liver lesion at department of Pathology, IGIMS, Patna, Bihar, India in between July 2015 to Jan 2017 . A detailed previous history of any other pre-existing liver disease and record of serological viral marker, where available, were collected from the surgery department. FNAC was carried out either

blindly or with USG/CT guidance in the radiology department. Direct air-dried smear was stained with MGG. Some smears were immediately fixed in 95% alcohol and stained with Pap. The remaining material in the syringe was allowed to clot to form cell block, where aspiration was adequate for cell block formation. Results were analyzed by two independent senior pathologists and a final conclusion of the diagnosis was derived after discussions with a third senior faculty. All the procedures were performed following the standard operating procedures with routine and consistent checks to identify and address various types of errors and omissions, ensuring data integrity, correctness and completeness of all the available records. The quality control checks included accurate patient identification, proper fixation time, adequate processing measures, appropriate embedding techniques, precision in microtome sectioning, unacceptable artifacts and regular inspection of controls used in IHC and special stains to determine the correctness in this method.

Statistical Analysis

Statistical analysis was done using Chi-square to compare various parameters. The P-value was calculated using the sampling distribution of the test statistics under the null hypothesis and our sample data as in a two-sided test. In our analysis, an alpha of 0.05 was used as the cut off for significance. When the P-value was less than 0.05, we rejected the null hypothesis that there is no difference between the means; thus, we concluded that a significant difference exists. So, in our study, P-value below 0.05 was taken as significant and over 0.05 as not significant. Fischer's exact test was also done to compare various parameters in the patients.

Results

Table 1: Demographic data

Gender	**N%**
Male	300 (60)
Female	200 (40)
Distribution of the cases in cell block preparation	
Metastatic	380 (76)
HCC	75 (15)
Suspicious of malignancy	10 (2)
Benign	35 (7)

There were 60% male and 40% females. Hepatocellular carcinoma was in the range of 46-82 years with a mean of 66.4 years while metastatic age range was 40-80 years with a mean of 58.2 years. On cell block, with or without immunohistochemistry, 75 cases (15%) were positive for hepato-cellular carcinoma, 380 cases (76%) were positive for metastatic lesions, 10 cases (2%) were suspicious of malignancy and 35 cases (7%) were designated as benign lesions.

Table 2: Correlation of the cases in cell block with that of biopsy with immunohistochemical markers, control used and source of origin of metastasis

Cell block			**Biopsy**	
HCC(75) Control-known HCC case	Poorly differentiated	2	HCC	2

Marker-Hep Par 1, pCEA, α feto protein	others	73		HCC	73
METASTATIC(380)	a)Adenocarcinoma Control-Appendix Marker-CK7, CK20,pCEA	Poorly differentiated	20	Adenocarcinoma Gall Bladder	4
				Adenocarcinoma others- Colon Stomach Ovary Pancreas HCC	 10 2 1 3 0
				HCC	0
		Well - mod differentiated	240	Adenocarcinoma others Colon Stomach Pancreas Ovary Unknown Primary	80 60 40 0 2
				Adenocarcinoma Gall Bladder	58
	b)undifferentiated Control-known poorly differentiated carcinoma Marker-CK7,CK20,pCEA,αfeto protein,SMA,HepPar1	70		Undifferentiated	50
				HCC	5
				Adenocarcinoma others	5
				SCC	4
				Sarcoma	6
	c)SCC Control-Seborrheic keratosis Marker-CK7,CK20	25		SCC	25
	d)Round cell Control-Ewings sarcoma Marker-Synaptophysin, Chromogranin	17		Round cell	18
	e) sarcoma Control-Fibroid Marker-SMA	8		Sarcoma	8

Suspicious of malignancy Control-All IHC-All	10		HCC	6
			Regenerative nodule	4
Benign (35)	Inflammatory	10	Round cell/Neuroendocrine tumor	2
			Hematological malignancy	2
			abscess	6
	Necrosis	7	Adenocarcinoma others	2
			Adenocarcinoma GB	2
			Abscess	3
	Benign	18	cirrhosis	10
			abscess	8

Individual comparison of cell block results with that of biopsy, which is the final diagnostic tool, showed a few discrepancies in interpretation of individual lesions.

Table 3: Differentiation of the tumors based on morphology

Morphology	**HCC**	**Poorly differentiated Metastatic carcinoma**	**Moderately differentiated to well differentiated metastatic carcinoma**	**Benign lesion**
1) Cytological pattern				
Trabecular pattern	++	+	-	+-
Hepatocytic appearance	+	+-	-	++
Intracellular bridge	+	+-	-	+-
2) Gland formation (in cell block /cytology)	+/-	+/-	+++	-
3) Special stains				
Reticulin stain	++	+-	-	+++/-
P & E	-	+/-	+-++	-

Morphology was observed from the smears obtained with MGG, PAP and H&E routinely from the cell block preparation. Special stain was PAS (to look for mucin) and reticulin (to look for trabecular strand) was also performed on cell block preparation.

Table 4: IHC study on the liver carcinomas

	Hepatocellular carcinoma	**Poorly differentiated metastatic Carcinoma**	**Moderately differentiated to well Differentiated metastatic carcinoma**	**Round Cell/ Neuroendocrine tumor**	**Sarcoma**	**Benign lesion**
CK7	-	+	++	-	-	-
CK20	-	+-	+	-	-	-
Hep Par-1	+	-	-	+-	-	+-
pCEA	+-	+-	+	-	-	-
α feto protein	++	+-	-	-	-	+-
Synaptophysin	--	+-	+-	+	-	-
Chromogranin	--	+-	+-	++	-	-
SMA	-	-	-	-	+	-

Table 3 and 4 were utilized to differentiate between hepatocellular carcinoma, poorly differentiated metastatic carcinoma, moderately to well differentiated metastatic carcinoma and benign lesions of the liver.

Table 5: Statistical analysis of the cell block and biopsy

	Analysis of all lesion in cell block with biopsy	**Analysis of hepatocellular carcinoma in cell block with biopsy**	**Analysis of metastatic carcinoma in cell block with biopsy**	**Analysis of different types of metastatic lesion in cell block with biopsy**	
Sensitivity	97.50%	92.8%	98.44%	Accuracy of Metastatic Adenocarcinoma	98.9% *P*<0.00001
				Accuracy of undifferentiated CA	100% *P*<0.00001
Specificity	84.36%	100%	96.0%	Accuracy of SCC	88.88% *P*<0.00001
Positive predictive value	98.0%	100%	98.2%	Accuracy of round cell carcinoma	85.7% *P*<0.00001
P-value	<0.00001	<.00001	0.00001	Accuracy of sarcoma	66.66% *P*<0.00640

A detailed statistical analysis showed sensitivity of all the lesions diagnosed through cell block method to be 97.50% with positive predictive value of 98% and P-value highly significant at <0.00001. Diagnosing metastatic carcinoma was also very accurate with positive predictive value of 98.2%. Primary lesion like hepatocellular carcinoma with 100% positive predictive value, 92.8% sensitivity and significant P-value had very precise results on cell block. However, differentiating the various types of metastatic lesions on cell block was less on target with accuracy ranging from 66.66% to 100% for various carcinomas.

Discussion

Intra-abdominal lesions possess significant diagnostic difficulties. Fine needle aspiration cytology (FNAC) plays an important role as it is rapid, less hazardous, and easy to perform diagnostic modality.[9] Imaging techniques such as ultrasonography, CT/MRI scan can be used as a guide for FNAC of intra-abdominal lesions by adding to the yield of the aspirate and providing important diagnostic clues.[10] However, FNAC sometimes does not yield information for precise diagnosis and there is always the risk of false negative/ indeterminate diagnosis. In these cases, cell block preparations can be helpful. Cell blocks are micro biopsies embedded in paraffin that broaden the diagnostic value of cytology specimens and compliments cytology smears. It employs retrieval of small tissue fragments from Fine needle aspiration specimens which are processed to form a paraffin block.[11] Liver shows an exhaustive gamut of pathology, focal and diffuse; benign and malignant; primary and metastatic. The metabolic functions of the organ and its dual vascular supply make the management of liver neoplasms a challenge. Accurate localization and characterization are pertinent for management decisions as they delineate the neoplasms that are compatible with surgical methods from those that need only palliative therapy. Radiological examination coupled with morphological assessment by fine needle

aspiration (FNA) cytology and/or lesional core biopsy (CB) is the first and pivotal step in this process.[12]

Tumor size (benign or malignant hepatic lesion) bigger than 5 cm had better successful aspiration and greater accuracy than tumor <1 cm. Similar results depending on tumor size is detected by Voit et al. and Willems et al.[13,14] There were 60% male and 40% females. Hepatocellular carcinoma was in the range of 46-82 years with a mean of 66.4 years while metastatic age range was 40-80 years with a mean of 58.2 years. The study by Mathew et al.[5] showed age range from 25-78 years with mean age at 58.5 years. The imaging results of most of the cases, comprising both hepatocellular carcinoma and metastatic lesion was a solitary SOL. Sukumaran et al[15] showed adeno-carcinoma to be the most common metastatic tumor at 83% followed by neuroendocrine tumor (15 cases), then poorly differentiated carcinoma with 1 or 2 cases each of other tumor like GIST, neuroblastoma, SCC and sarcomas. Our study follows the same trend of primary and metastatic carcinoma with mild variations in the unusual tumors' presentations. Cell block provides information like trabecular sinusoidal pattern, pseudo acini, arteries and absent reticulin framework which is adequate for differentiating well differentiated HCC from regenerating hepatocytes and also for differentiating poorly differentiated HCC from poorly differentiated metastatic carcinoma.

Metastatic cases in our study were the highest (76%) similar to Tao et al[16]whose study of 1037 cases showed 75% metastasis. In the present study, no recorded complications were present following FNAC, however, some authors have reported fatal bleeding in chronic liver disease, needle tract seedling and biliary venous fistula.[17,18] Intrahepatic hematoma was reported by Lundquist.[19] Careful histologic observations and judicious use of IHC acts as a useful adjunct in the right diagnosis of hepatic masses, highlighted in the study by Walther et al[20] CK7 and CK20 plays an important role in the diagnosis of metastatic carcinoma of unknown primary site. It provides diagnostic guidance in approximately 90% of undifferentiated malignant tumor though morphology also plays a fastidious role according to the study by Selves et al. and Fan et al.[21,22] Noh et al[23] found out in their study the relation between chronic HBV and HBC with the development of HCC. Zamor et al[24] believed HBV and HBC led to hepatic fibrosis which further developed into HCC. Their study showed 50% of cases were related to chronic hepatitis with majority residing in Asia.

In cell block, architecture of tumor is maintained at places whereas core biopsy can have crush artifact. Even in higher centers, in certain cases, cell block is better than core biopsy, which is formalin fixed, as studies show that formalin can hinder in DNA extraction, especially in molecular studies. However, in pediatric age group, FNAC with cell block can be used in certain cases though core biopsy remains the gold standard in most pediatric tumors.

Conclusion

A satisfactory FNAC sample with cell block is a very useful diagnostic tool for evaluation of various liver lesions with high degree of diagnostic accuracy. Also, it reduces the timing, the economic burden and morbidity of the patient. In cases where diagnosis by FNAC is equivocal, it is recommended to perform FNA with cell block preparation and IHC studies as a part of routine laboratory practice to improve diagnostic precision. Because of its high sensitivity, Cell Block technique is a useful adjunct to routine FNA smear because multiple sections can be cut from a cell block and IHC and special stains can be applied. Viral markers, if available, can be correlated to arrive at the final diagnosis. The combination of cell block

with all these adjunct techniques is of immense help in identifying primary carcinoma and differentiating it from metastatic deposits in the liver without any invasive procedure.

References

1. Mohmmed AA, Elsiddig S, Abdullhamid M, Gasim GI, Adam I. Ultrasound-guided fine needle aspiration cytology and cell block in the diagnosis of focal liver lesions at Khartoum Hospital, Sudan. Sudan Journal of Medical Sciences. 2012;7(3):183-7.
2. Khan AA, Jan GM, Wani NA. Fine needle aspiration of intra-abdominal masses for cytodiagnosis. Journal of the Indian Medical Association. 1996 May 1;94(5):167-8.
3. Zito FA, Gadaleta CD, Salvatore C, Filotico R, Labriola A, Marzullo A, Prete F, Marzullo F. A modified cell block technique for fine needle aspiration cytology. Acta cytologica. 1995 Jan 1;39(1):93-9.
4. Pillai S, Shanthakumari S. The significance of cell block and fine needle aspiration cytology in diagnosing a rare presentation of hepatocellular carcinoma. Indian Journal of Pathology and Microbiology. 2008 Jul 1;51(3):459.
5. Mathew EP, Nair V. Role of cell block in cytopathologic evaluation of image-guided fine needle aspiration cytology. Journal of cytology. 2017 Jul; 34(3):133.
6. Mallick S, Mallik M, Chatterjee RN, Chowdhury PS. Role of cell block technology as an adjunct to fine needle aspiration in evaluating as well as differentiating liver lesions. Iranian Journal of Pathology. 2021;16(4):392.
7. Anita AM, Mali MH, Patil AG, Meenakshi M. A comparative study of cell block and cytological smears in FNAC of intra-abdominal lesions. Journal of Pathology of Nepal. 2022;12(1):1900-6.
8. Koss LG, Melamed MR, editors. Koss' diagnostic cytology and its histopathologic bases. Lippincott Williams & Wilkins; 2006.
9. Aftab Khan A, Jan GM, Wani NA. Fine Needle Aspiration of Intra-abdominal masses for cytodiagnosis. J Indian Med Assoc. 1996;94(5):167- 9.
10. Zito FA, Gadaleta CD, Salvatore C, Filotico R, Labriola A, Marzullo A, Prete F, Marzullo F. A modified cell block technique for fine needle aspiration cytology. Acta cytologica. 1995 Jan 1;39(1):93-9.
11. Bales Carol E. Laboratory Techniques. In, Koss Leopold G. Koss' Diagnostic Cytology, 5th ed. Philadelphia: Lippincott Williams &Wilkins; 2006. 1856pp.
12. Soyuer I, Ekinci C, Kaya M, Bahar K. The value of fine needle aspiration biopsy in the diagnosis of metastatic liver tumours. Turk J Gastroenterol 2002; 13:78-82.
13. Voit CA, van Akkooi AC, Eggermont AM, Schafer-Hesterberg G, Kron M, Ulrich J, et al. Fine needle aspiration cytology of palpable and nonpalpable lymph nodes to detect metastatic melanoma. J Natl Cancer Inst. 2011;103(23):1771- 7.
14. Willems SM, van Deurzen CH, van Diest PJ. Diagnosis of breast lesions: fine-needle aspiration cytology or core needle biopsy? A review. J Clin Pathol. 2012;65(4):287-92.
15. Sukumaran RN, N, Katoor J, Sindhu Nair PA, KR Venugopal, M. Cytomorphological Spectrum of Ultrasound Guided Fine Needle Aspiration Cytology of Liver Lesions, and the Role of Cell Block Preparation and Immunohistochemistry in the Diagnosis: An Analysis of 638 Consecutive Aspirations. JMSCR 2008;6(4):875-81.
16. Tao LC, Donat EE, Ho CS, McLoughlin MJ. Percutaneous fine-needle aspiration biopsy of the liver.

Cytodiagnosis of hepatic cancer. Acta Cytol. 1979;23(4):287-91.
17. Mingoli A, Marzano M, Sgarzini G, Nardacchione F, Corzani F, Modini C. Fatal bleeding after fine-needle aspiration biopsy of the liver. Ital J Gastroenterol. 1995;27(5):250-1.
18. Patel RI, Shapiro MJ. Biliary venous fistula: an unusual complication of fine-needle aspiration biopsy of the liver. J Vasc Interv Radiol. 1995; 6(6):953-6.
19. Kuo FY, Chen WJ, Lu SN, Wang JH, Eng HL. Fine needle aspiration cytodiagnosis of liver tumors. Acta Cytol. 2004;48(2):142-8.
20. Walther Z, Jain D. Molecular pathology of hepatic neoplasms: classification and clinical significance. Patholog Res Int. 2011;403929.
21. Selves J, Long-Mira E, Mathieu MC, Rochaix P, Ilie M. Immunohistochemistry for Diagnosis of Metastatic Carcinomas of Unknown Primary Site. Cancers (Basel). 2018;10(4):108.
22. Fan Z, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. Mod Pathol. 2003;16(2):137-44.
23. Noh R, Lee DH, Kwon BW, Kim YH, Kim SB, Song IH. Clinical Impact of Viral Load on the Development of Hepatocellular Carcinoma and Liver-Related Mortality in Patients with Hepatitis C Virus Infection. Gastroenterol Res Pract. 2016; 2016:7476231.
24. Zamor PJ, deLemos AS, Russo MW. Viral hepatitis and hepatocellular carcinoma: etiology and management. J Gastrointest Oncol. 2017;8(2):229-42.