

## CYTODIAGNOSIS OF HEPATIC LESIONS AND THEIR ASSOCIATION WITH SERUM ALPHA FETOPROTEIN LEVELS

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### Abstract

Background: Liver is a common organ for lodgement of various infections and metastasis from other parts of body. Fine needle aspiration cytology (FNAC) plays a major decisive diagnostic role and combined with USG diagnostic accuracy is 90-95% minimizing the need of biopsy. An increased in serum AFP concentration has been observed in Hepatocellular Carcinoma (HCC) and is an important serum tumor marker. Methods: The present study was done, with the aim of studying the cytomorphological features of hepatic lesions and their association with serum Alpha Fetoprotein (AFP) levels. Study was conducted on 56 patients who underwent USG guided FNAC for diagnosis of liver lesions. Cytomorphological pattern of liver lesions were studied and serum AFP levels of the same cases were measured. Results: Maximum number of cases was in age group of 61-70 years. Male: Female ratio was 1.4:1. 82.1% cases were malignant. Most common malignancy was Metastatic deposits of Adenocarcinoma (MDA). HCC was diagnosed in 5.4% cases. Serum AFP levels in HCC were  $459.93 \pm 41.69$  ng/mL. 15.4 % of the cases of MDA and 8.3% of the cases of Metastatic deposits of Poorly Differentiated Carcinoma (MDPDC) showed raised serum AFP levels. Association between cytodagnosis and serum AFP range was statistically significant. Sensitivity=100%, specificity=81.4%, PPV=27.3% and NPV= 100% were obtained when serum AFP was used to differentiate metastatic lesions with HCC taking  $< 8.5$ ng/mL as cut off. Conclusion: USG guided FNAC of the liver is a rapid, reliable and economical screening test. Serum AFP has been the most widely used tumor marker for diagnosing HCC but raised levels are also seen in other hepatic lesions decreasing its specificity.

**Keywords:** Liver, FNAC, Serum Alpha fetoprotein levels

### Introduction

Liver is a vital organ which maintains the body's metabolic homeostasis.<sup>[1]</sup> Besides this, it is a common organ for lodgment of various infections and metastasis of tumors from various parts of the body especially the gastrointestinal tract.<sup>[2]</sup> The liver lesions includes primary liver neoplasms, metastatic deposits, congenital and acquired cysts, abscesses and granulomas.<sup>[3]</sup> Appropriate clinical management depends on accurate diagnosis of these lesions. Fine needle aspiration cytology (FNAC) plays a major decisive role and performing Ultrasonography (USG) guided FNAC helps to diagnose these lesions with 90-95% accuracy and minimizes the requirement of biopsy.<sup>[4]</sup> FNAC offers accuracy without significant

complication and requires minimal intervention, so warrants consideration early in investigative sequence.<sup>[3]</sup> Inflammatory lesions and diffuse liver diseases may mimic mass like lesions or appear as non-homogeneous lesions on radiological evaluation. Such lesions can also be sampled by FNA to rule out neoplasms and differentiate them from other benign and non- neoplastic lesions.<sup>[5]</sup> Alpha fetoprotein (AFP) is a major serum protein in the fetus, but the concentrations decrease rapidly after birth, and by the second year of life and after that only trace amounts are normally detected in serum. The reappearance of elevated AFP concentrations in adult serum has been observed in conjunction with various benign and malignant diseases.<sup>[6]</sup> AFP was first

described as a human tumor-associated protein and an increased serum AFP concentration in subjects with primary HCC was observed.<sup>[7]</sup>

#### Material and Methods:

The study was carried out over a period of 12 months from 1<sup>st</sup> February 2016 to 31<sup>st</sup> January 2017. Total of 56 cases which underwent image guided FNAC in Department of Radiology were received for cytology and were included in the study. Cases which already underwent treatment for any liver pathology or chemotherapy or radiotherapy for any illness were excluded from the study. The clinical history was recorded in the case reporting form. Image guided liver FNAC was performed on all patients by the radiologist after taking written consent. At least two wet fixed and two air dried smears were made and sent to cytopathology section of Pathology department. Cytomorphology was studied by using Papanicolaou (PAP) and Hematoxylin and Eosin (H&E) stain on wet fixed smears and May Grunwald Giemsa (MGG) stain on air dried smears and cytological findings were noted. Quantitative determination of serum AFP was done on all patients using VIDAS AFP kit. The statistical analysis was done using SPSS software (Statistical Package for Social Sciences) for Windows version 17.0.

#### Results:

Maximum number of cases were in age group of 61-70 years (n=19; 33.9%). The mean age was 59.21±13.24 years. Out of 56 cases, 58.9% of them (n=33) were males and 41.1% of the cases (n=23) were female. Male to Female ratio was found to be 1.4:1. Abdominal pain was the most common presenting complaints in 83.9% of the cases (n = 47), followed by generalized weakness (n=38; 67.9%). Only 28.6% of the cases (n=16) had jaundice as clinical sign. 46.4% of the cases (n=26) had history of alcohol abuse. 76.8% of the cases had multiple liver space occupying lesions on USG, while 23.2% of the cases had solitary lesions. 82.1% of the cases (n=46) had malignant liver lesions while 14.3% of the cases (n=8) were inconclusive. Only 1 of the case (1.8%) was benign. Diagnostic yield of USG guided liver FNAC was 82.1%. Table 1 shows that the most common cytomorphological diagnosis was of Metastatic deposits of Adenocarcinoma (MDA) (n=26; 46.4%) followed by Metastatic deposits of Poorly Differentiated Carcinoma (MDPDC) (n=12; 21.4%). In

MDA, cellular smear showed tumor cells arranged to form acini. Tumor cells were large, round to oval with pale eosinophilic cytoplasm. Nuclei having irregular nuclear membrane, coarse chromatin and prominent nucleoli (Figure 3). In MDPDC Cellular smear showed tumor cells which were markedly pleomorphic, showed high N:C ratio. Nuclei having irregular nuclear membrane and coarse chromatin (Figure 4). HCC was reported in 3 cases (5.4%). Cellular smears in these cases showed neoplastic cells forming widened trabeculae, multi-layered sheets. Small capillaries transgressing tumor cells' cluster (Figure 1). Polygonal cells with with increased nucleocytoplasmic ratio; tumor cells having round nuclei with coarse chromatin and central large macronucleoli (Figure 2). The benign group comprised of only 1 case which was reported as acute abscess. Cases having only benign reactive hepatocytes were categorized as inconclusive (n=8; 14.3%). Most common lesion among both males (n=14; 42.4%) and females (n=12; 52.1%) was MDA. HCC was seen only in males (n=3; 9.1%). Serum AFP levels were found to be higher when malignant lesions (55.04±133.14 ng/mL) were compared with benign lesions (1.69±0.00ng/mL) but no statistical significant difference observed. Maximum limit of serum AFP was 494.00ng/mL with mean value of 45.74±122.1 ng/mL. Serum AFP levels of the cases with HCC, MDA and MDPDC were found to higher than AFP values of Metastatic deposits of Small cell carcinoma (MDSCC), reactive hepatocytes (Table 2). Table 3 shows that 100% of the cases (n=3) of HCC had serum AFP levels of more than 400 ng/mL and none of other pattern showed this level. 15.4 % of the cases (n=4) of MDA and 8.3% of the cases (n=1) of MDPDC showed raised serum AFP with in the range of 8.6- 400 ng/mL. This association shows statistically significant difference between cytodagnosis and serum AFP range (p = 0.004). Table 4 shows that 88.3% of the cases (n=38) of metastatic hepatic lesions showed serum AFP levels < 8.5 ng/mL while none of cases of HCC showed serum AFP levels < 8.5 ng/mL. Five cases of metastatic hepatic lesions (11.7%) showed serum AFP levels > 8.5 ng/mL while all the 3 cases of HCC showed raised AFP levels. This association was found to be statistically significant (p = 0.004). Sensitivity, Specificity, PPV, NPV and Diagnostic accuracy of serum AFP for diagnosis of malignant hepatic lesions was 100.0%, 81.4%, 27.3%, 100.0% and 82.6% respectively.

**Table 1: Distribution of cases according to cytomorphological diagnosis (n=56)**

Cytomorphological diagnosis		Number of cases (n)	Percentage (%)
Malignant	HCC	3	5.4
	Metastatic deposits of adenocarcinoma	26	46.4
	Metastatic deposits of poorly differentiated carcinoma	12	21.4
	Metastatic deposits of small cell carcinoma	5	8.9
Benign	Acute abscess	1	1.8
Inconclusive		8	14.3
Inadequate		1	1.8
Total		56	100

**Table 2: Distribution of Serum AFP levels (ng/mL) in various Cytomorphological Diagnosis (n=56)**

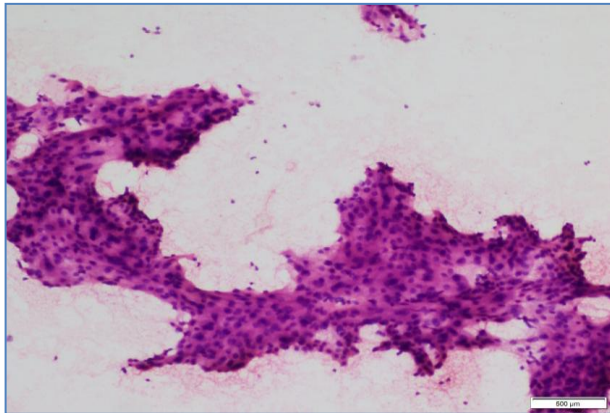
Cytodiagnosis	No. of Cases	Serum A.F.P (ng/mL)			
		Mean	S.D	Minimum	Maximum
Acute abscess	1	1.69	-	1.69	1.69
HCC	3	459.93	41.69	413.43	494.00
MDA	26	37.43	98.72	0.98	345.61
MDPDC	12	13.45	38.91	0.98	137.01
MDSCC	5	3.47	1.02	2.82	5.29
Inconclusive	8	3.09	1.78	1.06	6.41
Inadequate	1	3.13	-	3.13	3.13
Total	56	45.74	122.100	0.98	494.00

**Table 3: Association of cytomorphological diagnosis with serum AFP range (n=56)**

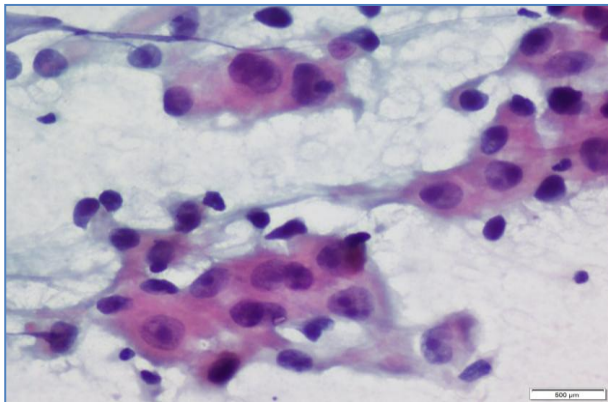
Cytodiagnosis	Serum AFP (ng/mL)						Total
	0-8.5		8.6-400		>400		
	n(48)	(%)	n(5)	%	n(3)	%	
Acute abscess	1	100	0	0	0	0	1
HCC	0	0	0	0	3	100	3
MDA	22	84.6	4	15.4	0	0	26
MDPDC	11	91.7	1	8.3	0	0	12
MDSCC	5	100	0	0	0	0	5
Inconclusive	8	100	0	0	0	0	8
Inadequate	1	100	0	0	0	0	1
Total	48	85.7	5	8.9	03	5.4	56

**Table 4: Association of Serum AFP levels in metastatic hepatic lesions and HCC (n=46)**

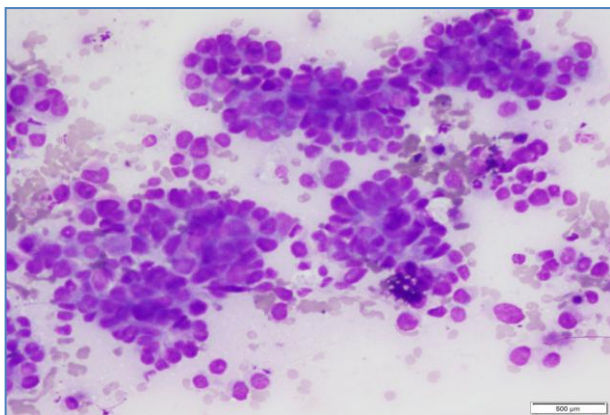
Serum AFP levels (ng/mL)	Metastatic Lesion		HCC	
	n	%	n	%
<8.5	38	88.3%	0	0.0%
>8.5	5	11.7%	3	100.0%
Total	43	100.0%	3	100.0%



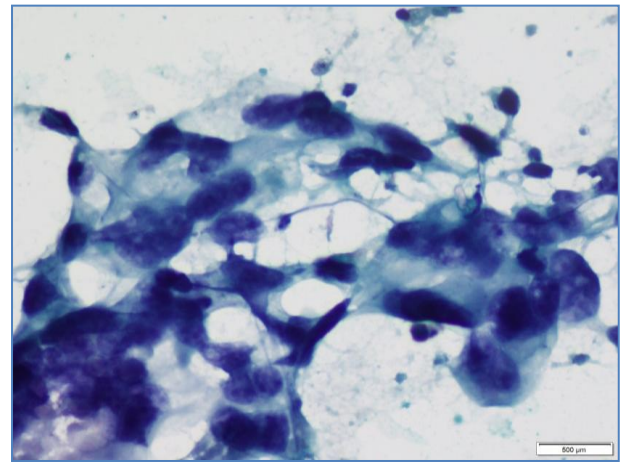
**Figure 1: HCC; Tumor showing presence of transgressing vessels (MGG Stain; 100X)**



**Figure 2: HCC; Tumor cells show coarse chromatin and prominent macronucleoli (PAP stain; 400X)**



**Figure 3: Metastatic deposits of adenocarcinoma; Tumor cells show acini formation (MGG Stain; 200X)**



**Figure 4: Metastatic deposits of poorly differentiated carcinoma; Tumor cells have pleomorphic nuclei with coarse chromatin (PAP Stain; 400X)**

**Discussion:**

Liver can be affected by a variety of diseases which are suspected clinically and biochemically. Radiologically, they present either as diffuse involvement of liver or as focal lesions and also designated as Space occupying lesions. FNAC has been proven as a very effective means of obtaining cells from many different body sites in various superficial as well as deep seated lesions for diagnosing pathology.<sup>[8]</sup> AFP can be expressed not only during periods of uncontrolled cell growth as in HCC but also after hepatic injury as in chronic viral hepatitis and cirrhosis.<sup>[9]</sup> In a study done by Kapadiya et al, age of cases with liver lesion ranged from 20 - 88 years and the maximum number of cases were in between 50-69 years.<sup>[10]</sup> Ali et al studied the role of FNAC in 138 cases of hepatic lesions with maximum number of cases between 40 to 55 years of age.<sup>[11]</sup> It was noticed that in most studies including the present one, maximum patients presented in 6<sup>th</sup> and 7<sup>th</sup> decade of their lives with liver lesions. This can be explained by predominance of malignant lesions in all the studies and most malignancies of liver present in elderly patients. There is a male predominance in most studies which is similar to our study where the male-to-female ratio was 1.4:1.

In various studies done by Ahuja et al,<sup>[12]</sup> Ali et al<sup>[11]</sup> and Abd-Elfatah et al<sup>[9]</sup> most common complaint was pain in abdomen which was concordant with the present study. In the present study showed that out of 56 cases, 28.6% of the cases had jaundice. These results were concordant with the study done by Alkarboly et al.<sup>[13]</sup> While in study conducted by Ahuja

et al only 12% cases presented with jaundice.<sup>[12]</sup> In study done by Sood et al 22.9% cases had history of alcohol abuse and considered it as an important etiological factor for various hepatic lesions.<sup>[14]</sup> In the present study, out of total 3 cases of HCC, 2 cases (67%) had history of alcohol abuse. While in study done by Arrieta et al, only 26% cases of HCC were alcoholics.<sup>[15]</sup> In the present study showed that 76.8% of the cases had multiple liver SOL's, while 23.2% of the cases had solitary lesion radiologically. These results are in line with study done by Alkarboly et al<sup>[13]</sup> and Agarwal et al.<sup>[16]</sup> Sood et al studied the profile of HCC and found multicentricity in 57% cases of HCC on USG.<sup>[14]</sup> While Pawlik et al found 82% of the cases of HCC to have solitary lesion.<sup>[17]</sup> In our study all the 3 cases of HCC had solitary lesion on USG. This supports the already established view that solitary lesions are more commonly found in cases of HCC while metastatic lesions most commonly present as multiple lesions.

In the present study 82.1% cases had malignant liver lesions and only 1 case (1.8%) had benign lesion. 8 (14.3%) cases were inconclusive which showed benign reactive hepatocytes and 1 case was inadequate which had only red blood cells. These results were concordant with the study done by Balani et al,<sup>[18]</sup> Agarwal et al.<sup>[16]</sup> Predominance of malignancy amongst liver lesions observed in our study can be explained by the fact that ours is a tertiary care hospital with Regional cancer research institute draining more of referred oncology cases. Also the cause of diagnosing increasing number of metastasis of primary tumor in liver could be due to late presentations to the hospital which can be attributed to lack of awareness and education and also due to lack of medical facilities in peripheral hilly regions of Uttarakhand. Our study showed that cytodiagnosis of only reactive hepatocytes was there in 14.3% of the cases (n=8). These lesions were neither categorised as malignant nor benign and are categorized as inconclusive aspirates on cytology. This may be due to an aspirate that obtained material only from the surrounding tissue of the tumor or missing the lesion, may show reactive and proliferative changes, whereas an aspirate from the centre of a large tumor may sample only degenerative and necrotic material. Therefore, for aspiration to be representative, the needle should pass through the entire mass of the lesion.

In study done by Agarwal et al in which out of 146 cases, metastatic tumors constituted 93.2% of

malignant cases and MDA was the commonest type. HCC was diagnosed in only 3 cases and lymphoma in 1 case.<sup>[16]</sup> Similar results were found in the study done by Balani et al<sup>[18]</sup> and in the present study. However these results were discordant with study done by Sattar et al who found nearly same distribution of cases of MDA (42.5%) and HCC (42.8%).<sup>[19]</sup> Nazir et al found that HCC was the most common lesion in 60.5% of the cases followed by metastatic deposits in 39.5% cases.<sup>[2]</sup> Early diagnosis and cytomorphological differentiation of Hepatocellular carcinoma from metastatic carcinoma is of special importance today because of feasibility of surgical resection in some early, selected cases. The most commonly encountered problem arises at the end of malignant spectrum i.e., distinguishing well differentiated from benign lesions and separating less differentiated from metastatic malignant lesions.<sup>[20]</sup>

The association between serum AFP and HCC has been widely examined and described by a large number of groups. But raised levels of serum AFP has also been found in various other hepatic lesions.<sup>[9]</sup> Hernandez et al found that serum AFP value was higher in HCC as compared to other lesions.<sup>[21]</sup> As shown in Table 2, AFP levels were higher in malignant lesions (55.04±133.14 ng/mL) as compared with benign lesions (1.69±0.00ng/mL) but no statistical significant difference observed between hepatic lesion and serum AFP levels. Elfatah et al found that 22.5% of the cases with benign liver lesions had mild elevation of serum AFP levels and concluded that elevated levels of AFP are not specific for HCC and can be seen in benign conditions like hepatitis C infection. AFP levels are useful as a non-invasive indicator of severity of liver dysfunction, degree of inflammation and stage of fibrosis.<sup>[9]</sup> Stuart et al studied the AFP levels in HCC cases and observed that 70% of the cases of HCC had an elevated AFP.<sup>[22]</sup> Similarly Alkarboly et al found 60% cases of HCC having raised AFP levels.<sup>[13]</sup> In the present study, all 3 cases of HCC had serum AFP levels of more than 400ng/mL. In present study out of 43 cases of metastatic carcinoma, 38 cases showed normal serum AFP levels while 5 cases of metastatic carcinomas showed raised serum AFP levels. These results were in line with study done by Alkarboly et al.<sup>[13]</sup>

The present study showed that out of 5 cases of metastatic carcinomas having raised AFP levels, maximum value of serum AFP (345.61ng/mL) was

seen with MDA followed by MDPDC (13.45±38.91 ng/mL) (Table 2). All the cases diagnosed as MDSCC, inadequate and inconclusive cases had serum AFP levels of less than 8.5ng/mL. Statistically significant difference was observed between cytodiagnosis of hepatic lesions and serum AFP range i.e < 8.5 ng/mL, 8.6 – 400 ng/mL and more than 400 ng/mL. The present study may suggest to consider serum AFP level of >400ng/mL as diagnostic cut off value for diagnosis of HCC. In the present study, 100% sensitivity, 81.4% specificity, 27.3% PPV and 100% NPV were obtained when serum AFP levels was used to differentiate metastatic hepatic lesions and HCC taking AFP level of <8.5ng/mL as cut off. The previous study conducted by Chan et al on 805 cases with hepatic mass showed the optimal cut-off AFP value was 10ng/mL, on which sensitivity, specificity, PPV and NPV were 82.6%, 70.4%, 86.6% and 63.6%, respectively. At the conventional cut-off value of 200ng/mL, sensitivity, specificity, PPV and NPV were 47.7%, 97.1%, 97.5% and 44.4%, respectively. For a definitive diagnosis of HCC (i.e. 100% specificity and PPV), the cut-off value was >500 ng/mL, with corresponding sensitivity and NPV of 38.1% and 41.1%, respectively.<sup>[23]</sup> In cases with strong clinico-radiological suspicion of malignancy and inconclusive FNAC, serum AFP which is minimally invasive investigation can be taken help of to support establishment of a presumptive diagnosis of HCC and strengthen the need for further work up of the patients, who might be lost to follow up after an inconclusive “only reactive hepatocytes” cytological diagnosis.

Limitation of the study are less number of benign cases as USG guided FNAC is mostly advised in malignant cases of focal hepatic lesions. Proving the non-malignant cases are true negatives with confidence is difficult because the original site of tumor might get missed during the procedure.

The present study concludes that USG guided FNAC of the liver is a rapid, reliable, accurate and economical screening test. It not only helps in differentiating between inflammatory, benign and malignant lesions, but also in categorization of different malignant lesions. The study concluded that hepatic lesions were more common in males, in the 6<sup>th</sup> and 7<sup>th</sup> decade of life with pain abdomen as the most common presenting complaint. MDA was found to be the most common malignant hepatic lesion among both males and females diagnosed on FNAC. Serum AFP levels were found to be diagnostic in HCC

cases as compared to other benign and malignant lesions. High levels of serum AFP were also seen in some cases of MDA and MDPDC which decreases the specificity of serum AFP levels in diagnosing HCC. Association between various hepatic lesions and serum AFP range was found statistically significant. The study concluded significant correlation of serum AFP levels in metastatic hepatic lesions and HCC. 100% sensitivity, 81.4% specificity and 82.6% accuracy of serum AFP in 56 cases of hepatic lesions was established. Although serum AFP has been the most widely used tumor marker for diagnosing HCC but raised levels are also seen in other hepatic lesions decreasing its specificity. This needs to be reviewed further and in larger study population.

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