

Research Article

A study of trucut biopsies of hepatic lesions with special reference to immunohistochemistry and special stain

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ABSTRACT

Background: Hepatocellular carcinoma is one of the most common malignancies that account to about half a million deaths yearly and is the third leading cause of death. 80% of these cases are seen in the Asian- Pacific region. Aims of the study were to study the role of immunohistochemistry in differentiating primary from metastatic neoplasms of liver and to evaluate the usefulness of reticulin in differentiating benign from malignant lesions of liver.

Methods: 46 cases of hepatic neoplasms reported were taken. Immunohistochemistry was done using the markers Alpha Fetoprotein (AFP), Hep Par 1, Cytokeratin 7 and Cytokeratin 20. Reticulin stain was done in 10 difficult cases to differentiate neoplastic from non-neoplastic lesions of liver.

Results: AFP was positive in 18 cases (84%), 20 cases were positive for Hep Par 1 (92%). Among the metastatic neoplasms, the most common expression was cytokeratin 7 positive/ cytokeratin 20 negative expressions which were observed in 54% of cases. Reticulin fibres in the benign processes revealed one-cell thick liver plates, whereas in dysplastic and carcinomatous deposits, they showed thickening of the hepatic cell plates which appeared as two or three cell-cell thick plates.

Conclusions: Alpha Fetoprotein and Hep Par 1 are found to be useful in diagnosing hepatocellular carcinomas; and Hep Par 1 is more sensitive than Alpha Fetoprotein. Cytokeratins 7 and 20 were useful in assessing the primary tumour to some extent in case of metastatic carcinomatous deposits of liver. If adequate liver biopsy sample is received, an extended panel of markers can be used to find the site of primary with more accuracy. Reticulin stain can be used in cases where there is difficulty in differentiating neoplastic from non-neoplastic lesions of liver.

Keywords: Hepatocellular carcinoma, Metastatic tumours, Immunohistochemistry, Reticulin stain

INTRODUCTION

Hepatocellular carcinoma is one of the most common malignancies that account to about half a million deaths yearly and is the third leading cause of death. 80% of these cases are seen in the Asian- Pacific region.

Only less than 20% of hepatocellular carcinomas are treated appropriately when they are diagnosed, due to the advanced stage of the disease at the time of diagnosis. The risk factors of this cancer are well known.

Worldwide, about 80% of cases are due to cirrhotic livers.² In Asia and Africa it is hepatitis B virus infection; In countries like Europe, Japan and United States, the bulk of the infection is due to hepatitis C than hepatitis B because of the vaccination given for newborns. Immunohistochemistry may help in distinguishing hepatocellular carcinoma and its mimics. The pivotal role of immunohistochemistry is in differentiating benign nodular lesions from reactive conditions, benign nodular lesions from well differentiated hepatocellular carcinoma, cholangiocarcinoma from metastasis and poorly

differentiated tumours of hepatic origin and to ascertain the origin of the tumour whether primary or secondary.

Alpha Fetoprotein is the protein that determines the histogenesis of the tumour from the liver. Bile canaliculi are stained by p-CEA and CD10. Hep Par 1 is a hepatocyte marker. CAM 5.2, Cytokeratin 8 and Cytokeratin 18 stain mature hepatocytes and nodules. Cytokeratin 7, Cytokeratin 19, Cytokeratin 20 and Cytokeratin AE1/AE3 are absent. Sinusoids take up CD34. Alpha Fetoprotein, p-CEA, CD10 and CD34 are the primary panel markers to tell that the malignant nodule is hepatocyte in origin. If histogenesis is questioned, then Hep Par 1 and Cytokeratin come into play.

The purpose of this study is to examine the usefulness of immunohistochemistry in differentiating primary from metastatic neoplasms of liver and to evaluate the role of reticulin stain to differentiate benign from malignant lesions of liver.

METHODS

Cases diagnosed as hepatic malignancy on liver biopsy specimens received in the Department of Pathology, Coimbatore Medical College, Coimbatore during a period from Jan 2012 to July 2013 were taken.

Inclusion criteria

- Liver biopsy specimens reported as dysplastic and neoplastic lesions of liver.
- Patient age more than 12 years.

Exclusion criteria

- Liver biopsy specimens other than dysplastic and neoplastic lesions of liver.
- Patient age less than 12 years.

Sections were cut at 4 microns thickness. Coated slides were used and the slides kept in incubation at 58 degrees overnight. The initial sections were stained with hematoxylin and eosin stain. The unstained slides were used for running reticulin stain by Gomori’s method and immunohistochemistry by a two-step indirect technique.

RESULTS

A total of 57 liver biopsies were reported in the Department of Pathology, Coimbatore Medical College over a period from Jan 2012 to July 2013. Out of these, 46 cases were reported as hepatic neoplasms in which 22 cases were reported as hepatocellular carcinomas and 24 cases as metastatic carcinomatous deposits in the liver.

In this study, all cases reported as hepatocellular carcinomas were stained immuno-histochemically with

Alpha Fetoprotein (AFP) and Hep Par 1. AFP was positive in 18 cases, giving a sensitivity of 84% (Figure 1). 20 out of 24 cases were positive for Hep Par 1 and thus the sensitivity of Hep Par 1 in this study was found to be 92% (Figure 2).

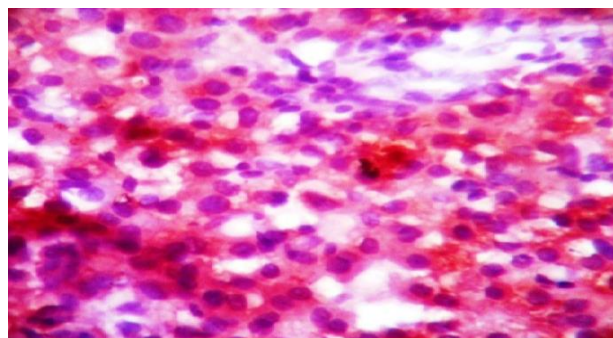


Figure 1: Immunohistochemical staining of hepatocellular carcinoma with alpha feto-protein. Most tumor cells express alpha feto-protein in the cytoplasm (40x).

So, it is observed that both alpha fetoprotein and Hep Par 1 are good immunohistochemical markers for the diagnosis of hepatocellular carcinoma; and Hep Par 1 is more sensitive than alpha fetoprotein (Figure 3).

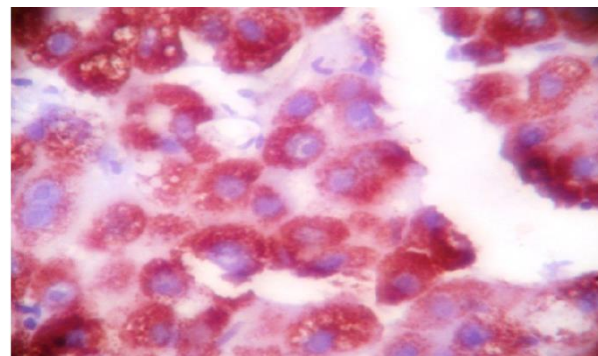


Figure 2: Immunohistochemical stain showing cytoplasmic granular positivity of Hep par1 in the tumor cells (40x).

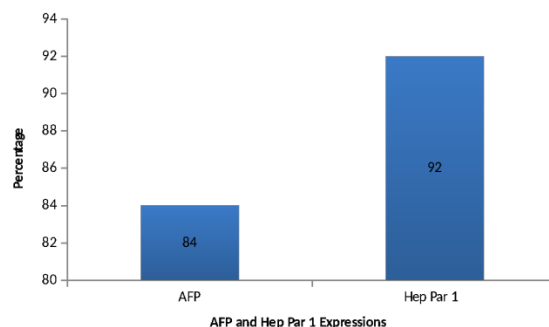


Figure 3: Percentage positivity of alpha fetoprotein (AFP) and Hep Par 1 expressions in hepatocellular carcinoma cases.

All 24 cases reported as metastatic carcinomas of liver were studied using the immunohistochemical markers Cytokeratin7 and Cytokeratin 20 (Table 1). From the study, the following observations were obtained. Number of Cytokeratin 7 positive and Cytokeratin20 negative cases: 13 (54%); Number of Cytokeratin 7 negative and Cytokeratin20 positive cases: 6 (23%); Number of Cytokeratin 7 negative and Cytokeratin20 negative cases: 6 (23%); Number of Cytokeratin 7 positive and Cytokeratin20 positive cases: 0 (0%).

Table 1: Expressions of cytokeratin 7 and cytokeratin 20 in metastatic carcinomas.

Markers	No. of positive cases	No. of negative cases	Percentage of positive cases (%)
Cytokeratin 7	7	6	54
Cytokeratin 20	3	10	23

Analysing the possible sites of primary tumour in these 24 cases of metastatic carcinomas (Table 2), in the study, the most common expression is cytokeratin 7 positive/ cytokeratin 20 negative, expression which is observed in 54% of cases, followed by cytokeratin7 negative/ cytokeratin 20 positive cases (23%), cytokeratin 7 negative/ cytokeratin 20 negative (23%) and cytokeratin 7 negative/ cytokeratin 20 negative (0%) expressions. So, the most common primary sites of tumours to metastasize to the liver are found to be from esophagus, stomach, breast, lungs, pancreas, biliary tract, ovary and endometrium.

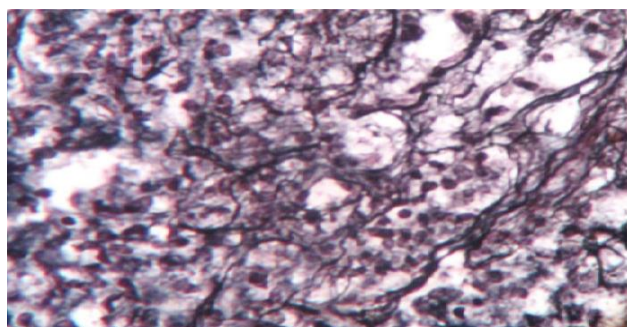


Figure 4: Well-differentiated hepatocellular carcinoma showing a compact pattern with in apparent sinusoids (reticulin stain) (40x).

At this point, it is worth mentioning about the size of liver biopsy specimens. The size of the liver biopsy specimens received in the laboratory during the period of the study was less than 1.5 cm which did not meet the required adequacy (at least 1.5 cm). So, only the more commonly used markers Alpha Fetoprotein, Hep Par 1, Cytokeratin 7 and Cytokeratin 20 could be studied.

Special stain with reticulin by Gomori’s method was done in 10 difficult cases to differentiate benign from malignant lesions. Reticulin fibres in the benign

processes revealed one-cell thick liver plates, whereas in dysplastic and carcinomatous deposits, they showed thickening of the hepatic cell plates which appeared as two or three cell-cell thick plates instead of usual one-cell thick plates (Figure 4).

Table 2: Patterns of cytokeratin expression in various types of metastatic tumours.

Cytokeratin 7 positive/ cytokeratin 20 positive tumours	Cytokeratin 7 positive/ cytokeratin 20 negative tumours
Urothelial carcinoma	Breast
Pancreas	Lungs
Biliary tract	Esophagus/stomach
Esophagus/stomach	Pancreas
Mucinous carcinoma (ovarian, colon, mucinous bronchoalveolar)	Biliary tract
	Ovary (nonmucinous)
	Endometrium
Cytokeratin 7 negative/ cytokeratin 20 positive tumours	Cytokeratin 7 negative/ cytokeratin 20 negative tumours
Colorectal	Prostate
	Renal cell carcinoma
	Adrenal cortical carcinoma

DISCUSSION

46 cases of hepatic malignancies during the period from Jan 2012 to July 2013 were studied. Statistical data of percentage positivity of Alpha Fetoprotein and Hep Par 1 in primary hepatic malignancies and Cytokeratin 7 and Cytokeratin 20 in metastatic carcinomas of the liver were studied. Among the 22 cases diagnosed as hepatocellular carcinoma, 20 cases (92%) were positive for Hep Par 1 which is comparable with other studies^{3,4} In the present study, 18 out of 24 cases of hepatocellular carcinomas stained positive for Alpha Fetoprotein; its sensitivity 84%.^{5,6} Thus, Hep Par 1 is more sensitive than Alpha Fetoprotein in the diagnosis of Hepatocellular carcinoma.

In the present study, cytokeratin 7 and cytokeratin 20 were positive in 13 and 6 cases respectively in the 24 metastatic carcinomas studied. Also, cytokeratin 7 positive/ cytokeratin 20 negative expression is the most common observation (54%) in this study which indicates that the most common tumours to metastasize to the liver are from esophagus, stomach, biliary tract, pancreas, breast, lungs, ovary and endometrium.⁵

Ideally a panel of markers including CD10, p CEA, Cytokeratin19, MOC-31 and villin should be used to diagnose hepatocellular carcinoma and to identify the primary site of origin of metastatic tumours with more accuracy; but only the more common and reliable markers Alpha Fetoprotein, Hep Par 1, Cytokeratin 7 and Cytokeratin 20 were included in the present study. This is because of the availability of very limited tissue in the biopsy specimen which warranted the judicious use of

markers. Special stain study with reticulin by Gomori's method was also done. It was found to be useful in differentiating benign from malignant lesions of liver.⁷

CONCLUSION

This is a retrospective study on hepatic neoplasms reported on liver biopsy specimens received in the Department of Pathology, Coimbatore Medical College, Coimbatore, Tamil Nadu, India over a period from Jan 2012 to July 2013.

46 out of 57 liver biopsy specimens received were reported as hepatic neoplasms; 22 cases were hepatocellular carcinomas and 24 were metastatic tumours in the liver. All 13 cases of hepatocellular carcinomas were studied using the immunohistochemical markers Alpha Fetoprotein and Hep Par 1. Alpha Fetoprotein was positive in 18 cases (84%) and Hep Par 1 was positive in 20 cases (92%).

Cytokeratin 7 and Cytokeratin 20 were studied in 24 cases of metastatic tumours. Majority of tumours showed Cytokeratin 7 positive/ cytokeratin 20 negative expressions (54%), which indicate that the most common primary sites were esophagus, stomach, pancreas, biliary tract, breast, lungs, ovary and endometrium. If adequate liver biopsy specimen is received (atleast 1.5 cm), an expanded panel of markers can be used to identify the primary site of tumour with more accuracy. Reticulin stain was also done in difficult cases to differentiate benign from malignant lesions of the liver, which was found to be useful.

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