ABSTRACT

DISSERTATION TITLE:

Role of IHC in differentiating primary hepatocellular carcinoma, cholangiocarcinoma & secondaries from colorectal region using manual tissue microarray technique & its advantages.

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AIMS AND OBJECTIVES

- To differentiate and to study the pattern of IHC markers like Hep-Par 1,CK7, CK19 and CK20 in primary hepatocellular carcinoma, cholangiocarcinoma and metastatic secondaries from colorectal region in liver.
- To demonstrate use of manual tissue microarray technique and its advantages of the same in IHC.

MATERIALS AND METHODS

The study was carried out in the Department of Pathology, Govt. Stanley Medical College, from July 2012 to June 2015 after obtaining the approval from Institutional Human Ethical Committee (IHEC) of Govt. Stanley Medical College, Chennai.

Total of 60 specimens were taken for this study.

INCLUSION CRITERIA

Histologically diagnosed cases of primary hepatocellular carcinoma, cholangiocarcinoma and metastatic secondaries from colorectal region in liver. (Trucut biopsies and resection specimens).

EXCLUSION CRITERIA

Benign tumours of liver, tumours of liver in infancy, mesenchymal tumours of liver.

METHODOLOGY

For all the 60 cases, details of age, sex and other relevant clinical data were recorded.

Microscopically diagnosed cases of primary hepatocellular carcinoma(well differentiated, moderately differentiated and poorly differentiated), intra hepatic cholangiocarcinoma and metastatic secondary deposits in liver from colorectal region in liver biopsies specimens were selected randomly irrespective of age and sex.

Method of data collection :

All Liver Biopsies and Resection specimens received in the Department of Pathology, Govt. Stanley Medical College were included in the study. 10% Neutral buffered formalin was used as fixative. Appropriate tissues were sampled and the tissues were processed in various grades of alcohol and xylol using automated histokinette. Paraffin blocks were prepared and sections of 5 micron thickness were cut and stained using H&

E technique and examined under the microscope for histopathological diagnosis and were taken up for the study(Using inclusion and Exclusion criteria). All the selected cases were included in the construction of tissue microarray.

Construction of Tissue Microarray

H & E slides were screened and the areas of interest were marked with marker pen which were again marked in the donor block. The recipient blocks were made by coring the paraffin block using 14 gauge bone marrow aspiration needles and the arrangement of the cores should be asymmetrical.

The cores from the donor block were taken from the areas of interest using 16 gauge needle. The diameter of the core was 1mm. These cores were placed in the recipient blocks as per our TMA design. This was placed in incubator at 37^{o}C for 24 hrs and kept in freezer compartment of refrigerator before sectioning. Each recipient block contains both controls and test tissue cores. The controls for Hep par1, CK7,19,20 are normal liver tissue, moderately differentiated gastric carcinoma, cholangiocarcinoma and moderately differentiated colonic adenocarcinoma respectively.

Sections were taken for IHC at 4 micron thickness in chrome alum coated slides using semi-automated microtome with disposable blades. The slides were kept in incubator at 70^{o}C for an hour.

Only 60 cases were studied in this study in view of less availability of carcinomas of liver in 2012- 2014.

Sections were subjected to antigen retrieval technique by pressure cooker method using TRIS EDTA (Ph 9) buffer solution and then treated by HRP (horse radish peroxidase) polymer technique.

EVALUATION OF IMMUNOSTAINING

Hep Par 1 – In this study we have used mouse monoclonal antibody which shows granular cytoplasmic positivity in immunostaining. The staining was observed in normal and neoplastic hepatocytes. The intensity of staining was scored[2] as-

0 = no reactivity;

1 = less than 5% of cancer cells positive;

2 = 5 - 25% positive;

3 = 25 - 50% positive,

4 = 50 - 75% positive;

5 = 75 - 90% positive; and

6 > 90% of tumour cells positive.

CK 7, 19, 20 - In this study we have used rabbit monoclonal antibodies which shows brown cytoplasmic and membranous staining. Positive immunoreactivity was defined as more than 20% of cells staining with the proper pattern of reactivity[40].

SUMMARY AND CONCLUSION

The study was carried out in the Department of Pathology, Govt.Stanley Medical College, over a period of three years from July 2012 to June 2015 after obtaining the approval from Institutional Human Ethical Committee (IHEC) of Govt. Stanley Medical College, Chennai.

1) Total number of cases studied were 60, which included 30 cases of Hepatocellular carcinoma(11-well differentiated,3-moderately differentiated,16-poorly differentited), 14 cases of Intrahepatic cholangiocarcinoma and 16 cases of Metastatic Adenocarcinomatous deposit in liver from colorectal region.

In this study we prepared manual tissue microarray blocks from the selected liver specimens fulfilling the inclusion and exclusion criteria.

TMA blocks are sectioned and Immunohistochemistry is done using Hep par1, CK 7, 19 and 20 to differentiate Hepatocellular

carcinoma, Intrahepatic Cholangiocarcinoma and Metastatic Adenocarcinomatous deposit in liver from colorectal region.

2) Incidence of HCC, ICC and Metastatic adenocarcinomatous deposit in liver fron colorectal region was high between 51 to 70 years.

3) Incidence of HCC was high compared other tumours.

4) There was only a single case of HCC below 30 years.

5) The mean age incidence of Hepatocellular carcinoma was 51.63 years, Intrahepatic cholangiocarcinoma was 57.43 years and Metastatic Adenocarcinomatous deposit in liver was 63.19 years.

6) Incidence of HCC was high in both genders.

7) Out of 30 cases of hepatocellular carcinoma, 11 were well differentiated, 3 were moderately differentiated and 16 were poorly differentiated hepatocellular carcinomas.

8) Out of 30 cases of Hepatocellular carcinoma, 24 cases were positive for Hep par1 and 6 cases were negative for Hep par1. Hep par1 was negative for 100% cases of Intrahepatic

Cholangiocarcinoma and Metastatic Adenocarcinomatous Deposit in liver from colorectal region.

9) CK 7 was positive in 100% cases of Cholangiocarcinoma, 3.33% 0f Hepatocellular carcinoma and 6.25% in Metastatic Adenocarcinomatous deposit in liver from colorectal region. CK 7 was negative in 96.67% cases of Hepatocellular carcinoma and 93.75% cases of Metastatic Adenocarcinomatous deposit in liver from colorectal region.

10) CK 19 was positive in 35.71% cases of Cholangiocarcinoma and 62.50% in Metastatic Adenocarcinomatous deposit in liver from colorectal region. CK 19 was negative in 100% cases in Hepatocellular carcinoma, 64.29% cases in Cholangiocarcinoma and 37.50% in Metastatic Adenocarcinomatous deposit in liver from colorectal region.

11) CK 20 was positive only in 87.50% cases of Metastatic Adenocarcinomatous deposit in liver from colorectal region. CK 20 was negative in 100% cases of Hepatocellular carcinoma and

Cholangiocarcinoma with 12.50% cases of Metastatic Adenocarcinomatous deposit in liver from colorectal region.

By the end of this study we conclude that manual TMA technique is superior to conventional technique of Immunohistochemistry and automated Micro arrayer instruments in terms of cost, time consumption, amount of reagents used and preservation of tissue of interest.

Using this panel of markers-Hep par1, CK 7,CK 19 and CK 20 we can differentiate Hepatocellular carcinoma, Intrahepatic Cholangiocarcinoma and Metastatic Adenocarcinomatous deposit in liver from colorectal region. The diagnosis of these malignancies is very important because the treatment protocols differ for each of these malignancies.