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Histopathological Analysis of Liver Biopsies and Hepatectomy Specimens with Cytokeratin 19 Study in Hepatocellular Carcinoma

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KEYWORDS

Hepatocellular carcinoma (HCC), Cytokeratin 19(CK 19), Liver Biopsies, Hepatectomy

ABSTRACT:

Background: Hepatocellular carcinoma (HCC) is a major global health burden with rising incidence and poor prognosis. Cytokeratin 19 (CK19), is a biliary/progenitor cell marker, has emerged as a potential indicator of tumor aggressiveness in HCC.

Objective: 1. To study patterns of hepatocellular carcinoma,2. Cytokeratin 19 expression in Primary Hepatocellular carcinoma to evaluate outcome, 3. To study adjacent liver changes noted in hepatocellular carcinoma proven hepatectomy specimens. Material and method: A prospective study was conducted in a total of 60 Liver carcinoma cases, during the period of December 2019 to November 2021 which we received biopsies and hepatectomy specimens were processed.

Results: Among the people with HCC diagnosis, 64.3% participants had CK 19. The difference in the proportion of CK 19 between HPE diagnosis was statistically significant (P value 0.040). Among the people with Clear Cell HCC diagnosis, 50% participants had Cirrhosis as Adjacent Liver Changes. The difference in the proportion of adjacent liver changes across HPE diagnosis was statistically not significant. (P value 0.724).

Conclusion: CK 19 Positive hepatocellular carcinoma is an aggressive subtype with early recurrence after hepatectomy, chemotherapy tolerance/local treatment and poor outcomes with worse overall outcomes. This study emphasizes the significance of CK19 as a prognostic biomarker in hepatocellular carcinoma.

INTRODUCTION

Cancer is one of the foremost health challenges faced worldwide, it accounts for a significant proportion of global mortality and morbidity. The second leading cause of death globally is due to cancer and it is responsible for nearly 10 million deaths annually, which is approximately one in six deaths worldwide [(1)]. India is currently facing a rapid increase in cancer burden, with the number of new cases estimated to upsurge from 1.46 million in 2022 to 1.57 million by 2025. In **2050**, the number of new incidence of cancer is projected to reach over **35 million** per year, a **77% increase** from 2022. The commonest cancers are lung cancer in men and breast cancer in women, while oral, breast, and cervical cancers are critical public health concerns, given their prevalence and late-stage detection rates[(2)].

Hepatocellular **cancer is the tenth most common cancer in India** with over **34,700 new cases and nearly 33,800 deaths annually.** Hepatocellular neoplasms are a group of heterogeneous disorders that includes benign, dysplastic and malignant lesions. Hepatocellular carcinoma (HCC) is the most common type of malignant lesion of the liver. Despite advances in diagnostic imaging, histopathological analysis of liver biopsies and hepatectomy specimens remains a cornerstone for definitive diagnosis, assessment of tumor grade, and identification of prognostic features in HCC[(3)].

Immunohistochemical markers have become crucial in the differential diagnosis of HCC from benign hepatic lesions and metastatic tumors. Among these markers, cytokeratin 19 (CK19), a type I intermediate

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filament protein commonly expressed in biliary epithelial cells and hepatic progenitor cells, has gained attention for its role in characterizing subgroups of HCC. The expression of CK19 is vanished in mature liver hepatocytes while it is constantly present in biliary epithelial cells and so CK19 became a significant marker of biliary epithelial cells in pathological diagnosis[3].

OBJECTIVES:

1. To study patterns of hepatocellular carcinoma
2. Cytokeratin 19 expression in Primary Hepatocellular carcinoma to evaluate outcome
3. To study adjacent liver changes noted in hepatocellular carcinoma proven hepatectomy specimens.

MATERIALS AND METHODS

A prospective study was conducted in a total of 60 Liver carcinoma cases, during the period of December 2019 to November 2021 which we received biopsies and hepatectomy specimens were processed. After diagnosis of hepatocellular carcinoma is made; the representative Paraffin Tissue block will be subjected to IHC for Cytokeratin 19 expression and reported from Department of Pathology, Stanley medical College. The study participants were selected using the following inclusion criteria

Inclusion criteria

- All cases of liver biopsies and hepatectomy specimens of hepatocellular carcinoma.

Exclusion criteria

- Benign Neoplasms of Liver
- Metastasis into the Liver
- Transplant liver

Methodology

Formalin fixed and paraffin embedded Liver cancer tissue blocks were selected in our Department of Pathology. Sections of 4-5µ thickness were cut from the paraffin block and taken on charged slides. Control block was selected. CK 19 – Cholangio-carcinoma, The CK 19 Immunohistochemical staining of representative paraffin tissue blocks done as per manufacturer guidelines. Immunohistochemically stained sections were examined under high power (40x) fields. CK 19 positivity considered as membranous positivity in tumor cells.

HRP Polymer Technique (Immunohistochemistry Method Using HRP-DAB Detection):

The HRP polymer technique involves a series of steps to ensure optimal staining and visualization of target antigens in tissue sections. Initially, the coated slides are incubated overnight at 37°C, followed by placement on a slide warmer at 70°C for 30 minutes on the day of the procedure. Deparaffinization is performed using two changes of xylene for 10 minutes each, and hydration is carried out with two changes of absolute alcohol, also for 10 minutes each. The slides are then washed with distilled water in two changes, 3 minutes each. Antigen retrieval is done using a pressure cooker: the retrieval buffer, TRIS-EDTA (pH 8.5–9.0), is preheated to 180°C for 5 minutes, and the slides are then processed in the cooker at 160°C for three whistles. After gradual cooling of the cooker, the slides are washed with distilled water (two changes, 3 minutes each) and TBS buffer (two changes, 3 minutes each).

To block endogenous peroxidase activity, hydrogen peroxide (H_2O_2) is applied for 10 minutes, followed by another TBS buffer wash. The primary antibody, Cytokeratin 19, is then applied and incubated for 45 minutes. After washing with TBS buffer, a target binder is added for 10 minutes to enhance the sensitivity of the antigen-antibody reaction, followed by another TBS buffer wash. The secondary antibody conjugated with horseradish peroxidase (HRP) is then applied for 10 minutes and washed off using TRIS buffer. DAB (diaminobenzidine) is prepared by mixing one drop of DAB chromogen with one ml of DAB buffer and applied for 5 minutes to visualize the antigen-antibody complex as a brown-colored precipitate at the site of binding. The slides are finally washed in distilled water, counterstained with hematoxylin for 30–60 seconds, air dried, and mounted with DPX (Dibutylphthalate Polystyrene Xylene) for microscopic evaluation.

RESULTS

Among the people with Clear Cell HCC diagnosis, 50% participants were aged between 31 to 40 years and remaining 50% participants were 51 to 60 years. Among the people with Fibrolamellar HCC diagnosis, overall 100% participants were aged between 11 to 30 years.

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Among the people with HCC diagnosis, 5.4% participants were 21 to 30 years, 7.1% participants were 31 to 40 years, 17.9% were 41 to 50 years,19.6% were 51 to 60 years, 37.5% were 61 to 70years, 8.9% were 71 to 80 years and only 3.6% participants were 81 to 90 years. The difference in the proportion of age across HPE diagnosis was statistically significant. (P value 0.003).

Table 1: Basic characteristics of the study population compared with HPE diagnosis

	HPE diagnosis			Total	p value
	Clear Cell HCC	Fibro-lamellar HCC	HCC		
Gender					
Male	1 (50%)	1 (50%)	53 (94.6%)	55 (91.7%)	0.008
Female	1 (50%)	1 (50%)	3 (5.4%)	5 (8.3%)	
Biopsy / Resection					
Biopsy	1 (50%)	1 (50%)	20 (35.7%)	22 (36.7%)	0.849
Resection	1 (50%)	1 (50%)	36 (64.3%)	38 (63.3%)	
LOBE with HPE diagnosis					
Both	0 (0%)	0 (0%)	5 (8.9%)	5 (8.3%)	0.535
Left	1 (50%)	2 (100%)	22 (39.3%)	25 (41.7%)	
Right	1 (50%)	0 (0%)	29 (51.8%)	30 (50%)	
Segment with HPE diagnosis					
2	1 (50%)	0 (0%)	13 (23.2%)	14 (23.3%)	0.496
3	1 (50%)	2 (100%)	11 (19.6%)	14 (23.3%)	0.020
4A	0 (0%)	0 (0%)	9 (16.1%)	9 (15%)	0.685
4B	0 (0%)	0 (0%)	6 (10.7%)	6 (10%)	0.788
5	1 (50%)	0 (0%)	11 (19.6%)	12 (20%)	0.443
6	0 (0%)	0 (0%)	14 (25%)	14 (23.3%)	0.521
7	0 (0%)	0 (0%)	12 (21.4%)	12 (20%)	0.585
8	0 (0%)	0 (0%)	5 (8.9%)	5 (8.3%)	0.823
Comparison of CK 19 with HPE diagnosis					
Negative	2 (100%)	2 (100%)	20 (35.7%)	24 (40%)	0.040
Positive	0 (0%)	0 (0%)	36 (64.3%)	36 (60%)	
Adjacent Liver Changes					
Cirrhosis	1 (50%)	0 (0%)	25 (44.6%)	26 (43.3%)	0.724
Steatosis	0 (0%)	0 (0%)	7 (12.5%)	7 (11.7%)	

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Steatosis with fibrosis	0 (0%)	0 (0%)	3 (5.4%)	3 (5%)	
Others	0 (0%)	0 (0%)	5 (8.9%)	5 (8.3%)	
Nil	1 (50%)	2 (100%)	16 (28.6%)	19 (31.7%)	
Total	**2 (100%)**	**2 (100%)**	**56 (100%)**	**60 (100%)**	

In all the three study groups, males outnumbered females. Among the HCC population, the proportion of males was 94.6%. The proportion of males was 50% among the clear cell HCC and fibrolamellar HCC. The differences across the HPE diagnosis in proportion of males and females was statistically significant (P value 0.008). Among the people with Clear Cell HCC diagnosis, 50% participants were biopsy and remaining 50% participants were resection. Among the people with Fibrolamellar HCC, 50% participants were biopsy and remaining 50% participants were resection. Among the people with HCC, 35.7% participants were biopsy and remaining 64.3% participants were resection. The difference in the proportion of biopsy / resection between HPE diagnosis was statistically not significant (P value 0.849).

Highest proportion of subjects with HCC (64.3%) had right lobe. This proportion was 50% among clear cell HCC population. The difference in the proportion of lobe between HPE diagnosis was statistically not significant (0535). Highest proportion of subjects with HCC (35%) were 6th segment. The difference in the proportion of 6th segment between HPE diagnosis was statistically not significant (0.521). The proportion of 3rd segment was 50%, 100% and 19.6% respectively among the moderately differentiated, poorly differentiated and well differentiated. The difference in the proportion of 3rd segment across grade was statistically significant. (P value 0.020). Among the people with HCC diagnosis, 64.3% participants had CK 19. The difference in the proportion of CK 19 between HPE diagnosis was statistically significant (P value 0.040). Among the people with Clear Cell HCC diagnosis, 50% participants had Cirrhosis as Adjacent Liver Changes. Among the people with HCC diagnosis, 44.6% participants had cirrhosis as adjacent liver changes, 12.5% participants had steatosis, 5.4% had Steatosis with fibrosis and 8.9% participants had others. The difference in the proportion of adjacent liver changes across HPE diagnosis was statistically not significant. (P value 0.724).

Table 2: Basic characteristics of the study population compared with HPE Grade

	Grade			**Total**	**p value**
	Moderately Differentiate d	**Poorly Differentiated**	**Well Differentiated**		
Gender					
Male	17 (85%)	2 (100%)	36 (94.7%)	55 (91.7%)	0.404
Female	3 (15%)	0 (0%)	3 (5.4%)	5 (8.3%)	
Biopsy / Resection					
Biopsy	6 (30%)	0 (0%)	16 (42.1%)	22 (36.7%)	0.363
Resection	14 (70%)	2 (100%)	22 (57.9%)	38 (63.3%)	
LOBE with HPE diagnosis					
Both	0 (0%)	0 (0%)	5 (13.2%)	5 (8.3%)	0.069
Left	5 (25%)	1 (50%)	19 (50%)	25 (41.7%)	

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Right	15 (75%)	1 (50%)	14 (36.8%)	30 (50%)	
Segment with HPE diagnosis					
2	3 (15%)	1 (50%)	10 (26.3%)	14 (23.3%)	0.415
3	3 (15%)	1 (50%)	10 (26.3%)	14 (23.3%)	0.415
4A	0 (0%)	0 (0%)	9 (23.7%)	9 (15%)	0.047
4B	1 (5%)	0 (0%)	5 (13.2%)	6 (10%)	0.549
5	2 (10%)	0 (0%)	10 (26.3%)	12 (20%)	0.260
6	7 (35%)	0 (0%)	7 (18.4%)	14 (23.3%)	0.267
7	6 (30%)	1 (50%)	5 (13.2%)	12 (20%)	0.175
8	2 (10%)	0 (0%)	3 (7.9%)	5 (8.3%)	0.876
CK 19 with grade					
Negative	2 (10%)	0 (0%)	22 (57.9%)	24 (40%)	<0.001
Positive	18 (90%)	2 (100%)	16 (42.1%)	36 (60%)	
Adjacent Liver Changes					
Cirrhosis	7 (35%)	2 (100%)	17 (44.7%)	26 (43.3%)	0.911
Steatosis	3 (15%)	0 (0%)	4 (10.5%)	7 (11.7%)	
Steatosis with fibrosis	1 (5%)	0 (0%)	2 (5.3%)	3 (5%)	
Others	2 (10%)	0 (0%)	3 (7.9%)	5 (8.3%)	
Nil	7 (35%)	0 (0%)	12 (31.6%)	19 (31.7%)	
Total	**20 (100%)**	**2 (100%)**	**38 (100%)**	**60 (100%)**	

Among the people with moderately differentiated grade, 5% participants were aged between 21 to 30 years, 71 to 80years, 81 to 90years and 15% participants were aged 31 to 40 years, 41 to 50 and 51 t 60. Among the people with poorly differentiated, 50% participants were aged between 41 to 50 years and 61 to 70. Among the people with Well Differentiated, 10.5% participants were 21 to 30 years, 5.3% participants were 31 to 40 years, 15.8% were 41 to 50 years,23.7% were 51 to 60 years, 31.6% were 61 to 70years, 10.5% were 71 to 80 years and only 2.6% participants were 81 to 90 years. The difference in the proportion of age across grade was statistically not significant. (P value 0.919). The proportion of male was 85%, 100% and 94.7% respectively among the moderately differentiated, poorly differentiated and well differentiated. The difference in the proportion of gender across grade was statistically not significant. (P value 0.404). The proportion of biopsy was 30% and 42.1% respectively among the moderately differentiated and well differentiated. The proportion of resection was 70%, 100% and 57.9% respectively among the moderately differentiated, poorly differentiated and well differentiated. The difference in the proportion of biopsy / resection across grade was statistically not significant. (P value 0.363). The difference in the proportion of lobe across grade was statistically not significant. (P value 0.069). The difference in the proportion of segment across grade was statistically not significant. (P value >0.05). The proportion of 4A segment was 23.7% respectively among the well differentiated. The difference in the proportion of 4A segment across grade was statistically significant. (P

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value 0.047). The proportion of CK19 was 90%, 100% and 42.1% respectively among the moderately differentiated, Poor and well differentiated. The difference in the proportion of CK 19 between grades was statistically significant (P value 0.001). The proportion of Cirrhosis was 35%, 100% and 44.7% respectively among the moderately differentiated, poor and well differentiated. The difference in the proportion of adjacent liver changes across grade was statistically not significant. (P value >0.05).

DISCUSSION

The present study confirmed a significant male preponderance (68%) among CK19-positive HCC cases, consistent with previous reports by Jian Yong Zhuo *et al.*, Xiabo Cai *et al.*, and Kaitlyn Kennedy *et al.*, which reported male proportions ranging from 71.4% to 78% [4] [5]. The protective role of estrogens and the tumor-promoting role of androgens in HCC progression further underscore the influence of sex harmones. The age distribution in our cohort showed a predominance in the >60 years group (68%), closely mirroring the 67% reported by Kaitlyn Kennedy *et al.* [6]. Our results support the concept that advanced age is a significant risk factor for HCC, likely due to cumulative liver insults from chronic hepatitis, metabolic syndrome etc.

The present study reported 60% CK19 positivity, notably higher than previously published rates: Wu *et al.* (27.24%), Lee *et al.* (30%), Miltiadous *et al.* (43.94%), Yang *et al.* (18.75%), and Fatourou *et al.* (10.11%) [7] [8]. This elevated rate may be attributable to methodological differences, population-specific factors, or poorly differentiated tumors in our cohort. CK19 expression, a marker of hepatic progenitor or cholangiocytic lineage, has been extensively associated with poor prognosis, early recurrence, microvascular invasion, and resistance to therapy. Yoshida *et al.* highlighted that CK19-positive residual tumors after incomplete RFA could be reactivated under hypoxic or thermal stress, indicating that CK19 not only marks aggressive tumors but also may mediate resistance to local ablative therapies so an aggressive or targeted therapies for CK19-positive tumors is warranted [9].

In our study, 44.6% of CK19-positive cases showed cirrhotic background, in agreement with Jian Yong Zhuo *et al.* (60.8%)(10). However, this proportion was lower than expected, and in some cases, steatosis or fibrosis without cirrhosis was observed. The majority of resected specimens were from moderately or poorly differentiated tumors, and CK19 positivity was significantly higher in resection specimens (64.3%). This may reflect a selection bias, as larger, resectable tumors tend to be sampled for CK19 expression more frequently.

CONCLUSION

CK19 is well recognized as a biliary/progenitor cell marker and a marker of tumor stem cell. CK 19 Positive hepatocellular carcinoma is an aggressive subtype with early recurrence after hepatectomy, chemotherapy tolerance/local treatment and poor outcomes with worse overall outcomes. This study emphasizes the significance of CK19 as a prognostic biomarker in hepatocellular carcinoma. CK19 expression was remarkably higher in male patients, older age groups, and in poorly differentiated tumors, and was often associated with cirrhotic liver changes. This study was consistent with global trends, though CK19 positivity appeared higher, possibly due to differences in tumor biology or patient selection. Given its routine CK19 evaluation in HCC patients showed strong association with aggressive histology and poor outcomes, which may help stratify risk, to decide on treatment protocols, and to plan the follow-up strategies. Regular follow up of patient with history of HCC for CK19 can be used for early detection of recurrence thus reducing the mortality and morbidity.

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