Pattern of Different Immunohistochemical Stains in Various Types of Hepatoblastoma

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ABSTRACT

Objective: To assess the utility of four immunohistochemical stains in the categorization of hepatoblastoma.
Study Design: Retrospective longitudinal study.
Place and Duration of Study: Histopathology Department, Shaukat Khanum Memorial Cancer Hospital, Peshawar Pakistan, from 2016 to 2018.
Methodology: We retrospectively reviewed 50 cases of hepatoblastoma from 2016-2018, from our hospital database and studied the pattern of four immunohistochemical stains (Beta-catenin, Glutamine synthetase, Heppar 1 and Cyclin D1) in various subtypes.
Results: Ten out of fifty cases were mixed epithelial and mesenchymal hepatoblastomas, and forty were pure epithelial. In the pure epithelial category, the fetal subtype showed Heppar 1 and Cyclin D1 positivity in twenty-eight cases, while Glutamine synthetase and Beta-catenin were positive in all the thirty cases. One case of embryonal subtype displayed negativity for Heppar 1; the rest of the immunohistochemical stains were positive. Ten tumours exhibiting mixed epithelial and mesenchymal morphology showed positivity for all the four immunohistochemical stains in the epithelial component. However, Heppar 1 and cyclin D1 were negative in the mesenchymal component in all the ten cases. Five cases (50%) showed Glutamine synthetase and Beta-catenin positivity in the mesenchymal component.
Conclusion: All the four immunohistochemical stains, especially Beta-catenin and Glutamine synthetase, were efficient diagnostic tool, especially for tumours with complex or vague morphological features.

Keywords: Hepatoblastoma, Immunohistochemical stains, Liver neoplasm.

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INTRODUCTION

Hepatoblastoma is the most common paediatric liver malignancy accounting for 66% of the malignant hepatic neoplasms in childhood and is usually diagnosed by the age of three years. Hepatoblastomas are classified as epithelial and mixed types.[1,2,3] Epithelial hepatoblastoma is further divided into fetal, embryonal, macrotrabecular, small cell undifferentiated and cholangioblastic types. Mixed hepatoblastoma (mixed epithelial and mesenchymal) is further subdivided based on the presence or absence of teratoid features.[5,6]

These subtypes can be determined based on distinct morphological features. However, immunohistochemistry can aid in the diagnosis of cases with indeterminate morphology. Prognosis is based on numerous factors, including age at the time of diagnosis, metastases, alpha fetal protein (AFP) levels, histologic subtype, completeness of resection, and clinical stage of the disease.[7,8] As prognosis and treatment may differ according to the morphological subtype, the use of immunohistochemistry in future may improve standardization of treatment stratification according to tumour histopathology.[9,10]

The objective of our study was to evaluate the expression of four antibodies in hepatoblastoma, i.e. Glutamine synthetase, Heppar 1, Beta-catenin and Cyclin D1.

METHODOLOGY

We retrospectively reviewed 50 cases of hepatoblastoma, from 2016-2018, at Shaukat Khanum Memorial Cancer Hospital and Research Centre, Peshawar. Ethical approval of the study was obtained from our Internal Review Board (EX-02-08-19-09).

Inclusion Criteria: All the cases of hepatoblastoma diagnosed on the core and excision specimens were included in the study.

Exclusion Criteria: All the cases with prior neoadjuvant chemotherapy, radiotherapy or combined chemoradiotherapy, specimens with poor fixation or processing artefacts and presence of extensive necrosis and autolysis were excluded from the study.

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Hematoxylin and eosin-stained slides and antibody stained slides of 4-5 microns thick sections were prepared using Leica Peloris for processing, Thermo Histostar for Embedding, Leica RM 2245 for microtomy, Leica ST 5020 for staining and Leica CV 5030 for coverslipping. The expression of four antibodies was analyzed in different subtypes of the tumour. The names of antibodies with clones used, antibody incu-bation and retrieval times were shown in the Table.

Table: Names of antibodies with clones used, antibody incubation and retrieval times

Antibody Time	Clone	Antibody Incubation Time	Antigenre-trival Time
Beta-catenin	17C2	15 minutes	20 minutes
Heppar 1	Och1e5	16 minutes	36 minutes
CyclinD1	EP12	15 minutes	40 minutes
Glutamine synthetase	GS-6	15 minutes	40 minutes

Statistical Package for Social Sciences (SPSS) version 20 was used for the data analysis. Age of the patients, gender, type of hepatoblastoma and staining patterns were evaluated.

RESULTS

Fifty cases of untreated hepatoblastoma were evaluated. Age of the patients ranged from 1 month to 12 years with the mean age of 4.1 ± 1.4 years. Eight patients were females and forty two patients were males (Male:female = 5:1). Ten out of fifty cases were mixed epithelial and mesenchymal hepatoblastomas and forty were pure epithelial. The epithelial tumors were of fetal epithelial subtype in thirty cases and embryonal epithelial subtype in two cases. Eight cases displayed mixed fetal and embryonal morphology.

In pure epithelial category, the fetal subtype showed Heppar 1 and Cyclin D1 positivity in twenty eight cases (93%) while both Glutamine synthetase and Beta catenin were positive in all the cases 30 (100%). One case (50%) in embryonal subtype displayed negativity for Heppar 1, rest of the immunohistochemical stains were positive. Of eight cases showing both fetal and embryonal epithelial components all the stains were positive except two cases showing negativity for Cyclin D1 (75% positive). One case in the mixed category although positive for Heppar 1 showed staining only in fetal component (Figure-1A & 1B).

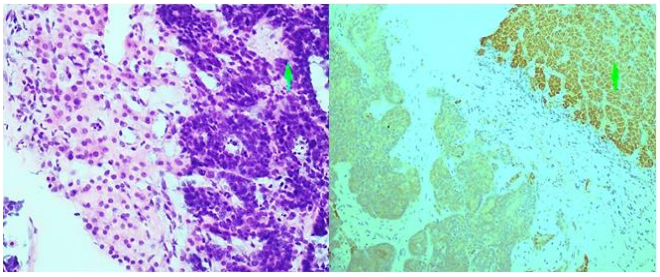

Figure-1A & 1B: Epithelial hepatoblastoma with both fetal and embryonal components and Heppar 1 stain, which in this particular case only stained the fetal component.

In 50% cases, Beta catenin also showed cytoplasmic in addition to nuclear staining (Figure-2A & 2B). Beta catenin normal membranous staining pattern was observed in the background hepatocytes in a few cases.

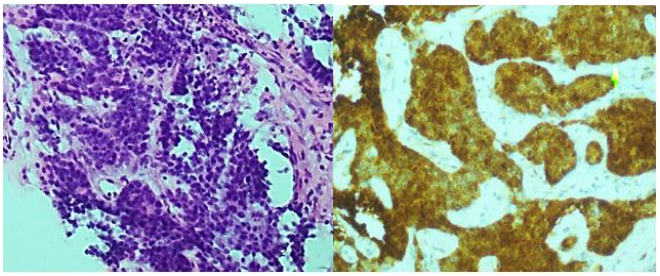

Figure-2A & 2B: Pure embryonal type hepatoblastoma with angulated, hyperchromatic nuclei and scant cytoplasm. Beta catenin staining in embryonal hepatoblastoma with cytoplasmic and nuclear staining pattern.

Ten tumors exhibiting mixed epithelial and mesenchymal morphology showed positivity for all the four immunohistochemical stains in the epithelial component. However, Heppar 1 and Cyclin D1 were negative in mesenchymal component in all the ten cases (0%). Five cases (50%) showed Glutamine synthetase and Beta catenin positivity in the mesenchymal component. Pictorial representation is given in Figure-3A-3D.

In all the cases positive for Cyclin D1 , the percentage of positive cells ranged from 40% to 70% instead of other stains that stained more than 80% cells when positive.

DISCUSSION

Our study evaluated the expression of Heppar 1, Glutamine synthetase, Beta-catenin and cyclin D1. Hopper 1 is usually present in epithelial hepatoblastoma with negative expression in some embryonal subtypes. It is always negative in mesenchymal areas and small-cell undifferentiated types.

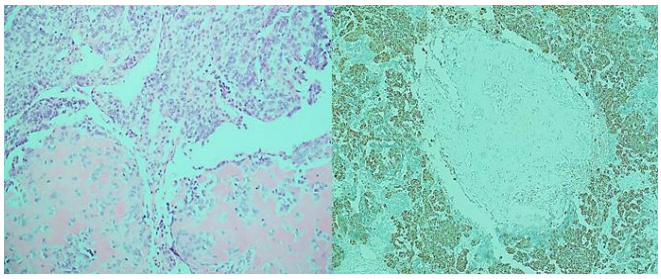

Figure-3A: Mixed epithelial and mesenchymal hepatoblastoma (with osteoid production). Figure-3B: Heppar 1 which WAS staining only epithelial component.

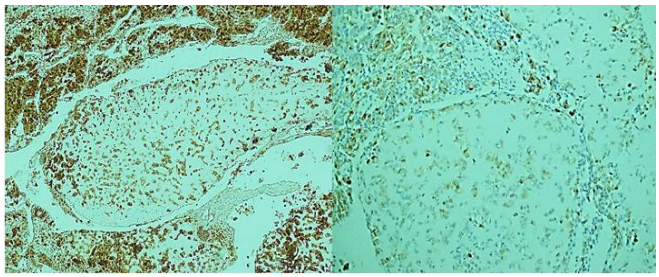

Figure-3C & 3D: Beta catenin (left) and glutamine synthetase (right) staining in both epithelial and mesenchymal components.

Beta-catenin is a marker of the activated Wnt pathway. CTNNB1 activating mutations are found in >80% of hepatoblastoma cases. Although the nuclear expression is more specific, diffuse cytoplas-mic staining is also seen in neoplastic cells. Glutamine synthetase is a downstream target of the Wnt/Beta-catenin pathway.[11,12] Normal Glutamine synthetase staining is seen in a single layer of normal hepatocytes around the central vein. Cyclin D1 is a relatively less commonly used marker, which shows staining in fetal, embryonal and small-cell undifferentiated subtypes with variable staining in mesenchymal components.[13,14]

Fasano *et al,* studied the expression of Heppar 1 in 12 cases of hepatoblastoma regardless of tumour subtype and in 27 other paediatric malignancies. All the twelwe cases showed cytoplasmic expression for Heppar 1, and other paediatric malignancies were negative except for a single case of hepatocellular carcinoma.[7] Our study also demonstrated that most of the cases of hepatoblastoma express Heppar 1, i.e. 28 of 30 cases with fetal morphology were positive, and one case with embryonal morphology was positive. The mesenchymal component in the mixed subtype was invariably negative.

Beta-catenin and Glutamine synthetase were associated with the same pathway and have been studied as potential diagnostic markers in diagnosing hepatoblastoma.[8,9,10,15] Huang *et al,* evaluated Beta-catenin and Glutamine synthetase staining in 18 untreated and 22 post-treatment cases. Their study concludes that both these markers were beneficial in the pretreatment group and post-treatment specimen.[8,17] tumours were positive for Beta-catenin in both epithelial and mesenchymal components in the pretreatment group. The epithelial component of all the untreated cases showed intense cytoplasmic Glutamine synthetase staining, and malignant mesenchymal elements in all the cases were negative.[8] Similarly, in our study, strong diffuse nuclear and cytoplasmic beta-catenin staining in all cases with epithelial morphology. However, only 50% of cases with mesenchymal components were stained with both Beta-catenin and Glutamine synthetase. Wu *et al,* studied immunohistochemical staining of 31 pretreatment tumour specimens. Nuclear and cytoplasmic staining of Beta-catenin was seen in 27 patients and was negative in 4 patients regardless of the epithelial or mesenchymal components.[13] Our study demonstrated a difference in mesenchymal component, where only 50 percent (5/10) were positive, epithelial component showed positivity in 100% cases.

Anna *et al,* studied the expression of Cyclin D1 in 5 cases of hepatoblastoma by Western analysis and immunohistochemistry. Both studies had similar results showing strong expression in all the cases regardless of tumour morphology.[14] In our study, however, the mesenchymal component was negative of 30 cases with fetal morphology, two were negative. Out of eight cases with both embryonal and fetal morphology, two were negative. This gave us a combined percentage of 84.15% expression in hepatoblastomas. Cyclin D1 was one of the first genes to be implicated as a target gene of the activated canonical Wnt pathway. Koch *et al,* studied the expression of cyclin D1 in 23 cases of hepatoblastomas by RT-PCR, with increased expression observed in 12 cases.[15] Kim *et al,* reported the increased expression of cyclin D1 in 13 cases (76%) of hepatoblastomas,[16] which was almost in concordance with our percentage of 84.15% in epithelial hepatoblastoma. Our study was in accordance with many other studies done on staining patterns in hepatoblastoma worldwide.[17,18]

CONCLUSION

All the four immunohistochemical stains, especially Beta-catenin and Glutamine synthetase, were efficient diagnostic tool, especially for tumours with complex or vague morphological features.

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Conflict of Interest: None.

Authors' Contribution

HM: Data analysis write up, SM: Supervisor, design review, MH:, UNS: Review, UH: Design, SS: Interpretation, MH:, MTM:, AHA: Review.

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