Utility of Cytokeratin 7 Immunocytochemistry in the Cytopathological Diagnosis of Fibrolamellar Hepatocellular Carcinoma

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Abstract

Objective: To distinguish fibrolamellar hepatocellular carcinoma (FL-HCC) variant from the conventional hepatocellular carcinoma (HCC) by cytology, immunocytochemistry, and morphometry. Study Design: Retrospective detailed cytomorphological, immunocytochemical, and morphometric analysis was performed in 6 cases of FL-HCC reported on fine needle aspiration. Cell block immunocytochemistry (CB-ICC) for CK7 and CD68 was performed in four cases. Morphometry was carried out with Cell A software. Area of the cell, nucleus and nucleolus was measured in 50 nuclei per case in 6 cases each of FL-HCC and HCC. Results: The mean age of patients with FL-HCC was 19 years and all had normal serum alpha-fetoprotein levels. Fine needle aspiration smears showed large polygonal cells with abundant cytoplasm, vesicular nucleus and prominent nucleolus, associated with variably cellular fibrous stromal fragments. Intranuclear inclusions, cytoplasmic eosinophilic inclusions, and bile were also noted. FL-HCC showed strong membrano-cytoplasmic CK7 positivity and cytoplasmic granular and canalicular positivity for CD68. In contrast, HCC showed weak focal positivity for CK7 and only canalicular CD68 positivity. Morphometry revealed that FL-HCC cells were 2.19 times the size of HCC. Conclusion: CK7 immunocytochemistry on cell blocks is useful for confirming and distinguishing it from HCC.

Keywords: Cytokeratin 7, fibrolamellar hepatocellular carcinoma, fine needle aspiration, immunocytochemistry, morphometry

Introduction

Hepatocellular carcinoma (HCC) is one of the most common solid tumors of adult human males in the world, with marked geographical differences in its prevalence. It carries a poor prognosis, with survival measured in weeks or months.[1–3] An unusual variant, characterized by a better prognosis and a distinctive histologic pattern, was described by Edmondson in 1956;[4] it was not until the 2010 edition that this entity “Fibrolamellar hepatocellular carcinoma (FL-HCC)” was given its own classification number. FL-HCC typically shows presence of a liver space occupying lesion with normal serum alpha-fetoprotein (AFP) levels.[5,6] As per the SEER report, patients with FL-HCC are more likely to receive surgery as a form of treatment and have a better five-year survival as compared to conventional hepatocellular carcinoma (HCC).[7] In addition to surgery, these patients are also more frequently candidates for radioablation and transplant and hence their distinction from HCC is important. Fine needle aspiration cytology (FNAC) is often performed for liver space occupying lesions, especially in those cases with normal serum alpha-fetoprotein and lack of associated cirrhosis. Hence, it is important to be aware of the spectrum of cytomorphology in FNAC smears. In this report, we have for the first time performed immunocytochemistry for cytokeratin 7 (CK7) and CD68, putative markers for this neoplasm, on cell blocks and compared the expression in conventional HCC to evaluate their utility in cytopathological diagnosis of FL-HCC.

Materials and Methods

Six cases of FL-HCC diagnosed between 2008 and 2015 were retrieved from the archives of the Department of Cytology. All the patients had undergone ultrasound-guided fine needle
aspiration using 23 G spinal needles with a stylet. A confirmed diagnosis of FL-HCC was given in all six cases on cytology smears and the hematoxyline-eosin and May-Grünewald Giemsa stained smears were available for review and morphometry. All cases had well-preserved slides, optimal for evaluation, featuring abundant malignant liver cells.

**Immunocytochemistry (ICC)**

ICC was performed on cell blocks of the aspirate which was available to us in four cases of FL-HCC and they were compared with four cases of HCC. Immunostaining was performed using primary antibodies against CK7 (clone OV-TL, Dako M701, 1:200 dilution) and CD68 (clone PG-M1, Dako M0876, 1:100 dilution) using the automated immunostainer (Ventana-Roche, USA).

**Morphometry**

Morphometry was carried on all 6 cases of FL-HCC and compared to 6 cases of well-differentiated HCC retrieved from our archives. All these 6 cases of HCC had markedly raised serum AFP levels, with classical radiological features of HCC. The measurements were performed using an Olympus BX53F Microscope (Tokyo, Japan) with Cell A software. One well spread MGG-stained slide was selected in each case and 50 isolated neoplastic cells with well-defined cytoplasmic borders were measured in each case. The parameters measured were total area of the cell, nucleus area, and the nucleolar area. Briefly, the area to be measured was outlined using the freehand tool and the area in µm² was displayed on-screen. The nuclear/cytoplasmic (N/C) ratio was obtained by dividing the respective areas.

**Statistical tests**

To test the significance of relationship between case and controls (test control), linear regression model was applied to look at the coefficient of determination. For each of these models we also verified whether the $P$ values of the regression coefficients were significant.

**Results**

All six cases diagnosed as FL-HCC in this series were young patients with age ranging between 7 and 24 years (mean 19 years), and all had normal serum AFP levels. There were 5 males and 1 female. The size of the tumors ranged from 2–13 cms in maximum dimension. There was no evidence of associated cirrhosis in any case. In two cases, there was evidence of hilar lymph nodal metastases confirmed by fine needle aspiration.

**Cytomorphology**

All the aspirates were moderate to highly cellular. There were loose aggregates [Figure 1a] and many isolated large discohesive cells which were intermixed with fibrous stromal fragments [Figure 1b]. The cells had abundant eosinophilic cytoplasm with well-defined boundaries. Nuclear pseudoinclusions [Figure 1c], intracytoplasmic pale bodies, hyaline or eosinophilic inclusions [Figure 1d] and bile pigment [Figure 1e] were seen in variable quantities in every case. Most of the cells had a large central nucleus displaying a huge nucleolus. Binucleation was present in all cases. Multinucleation was also noted [Figure 1e]. Similar features were seen in section from cell block [Figure 1f]. The detailed case by case cytological features are tabulated in Table 1.

**Immunocytochemistry**

Immunocytochemistry was performed on cell blocks. FL-HCC showed strong cytoplasmic and membranous CK7 positivity [Figure 2a] in contrast to HCC, which showed very focal and weak CK7 positivity [Figure 2b]. CD68 immunostaining revealed focal cytoplasmic granular along with canalicular positivity for CD68 in FL-HCC [Figure 2c] and a predominantly canalicular positivity in HCC, NOS [Figure 2d]. HepPar1 was not discriminatory between the two entities.

**Morphometric analysis**

In order to quantify the morphological observations, morphometry was carried out and analysis is detailed in Table 2. FL-HCC tumor cells showed mean 1100 µm² cell area, mean nuclear area of 190 µm² and a mean nucleolar area of 33 µm². In contrast, the mean cellular, nuclear, and nucleolar
area of tumor cells in selected cases of well differentiated HCC was 489 µm², 95 µm², and 10 µm², respectively. Nucleoli were not prominent in all cells in HCC; so nucleolar area in HCC could not be measured in all cells. A few ‘normal’ hepatocytes admixed with tumor cells were also measured for comparison and showed mean cellular and nuclear area of 363 µm² and 53 µm², respectively. The N/C ratio of HCC ranged between 0.065–0.391 (mean 0.202); the N/C ratio of FL-HCC ranged between 0.085–0.357 (mean 0.182). On applying linear regression model, we obtained the coefficient of determination close to one for all the variables. This indicates that the models used for assessment are significant. Also, the sensitivity values/coefficients were found to be significant with a level of significance ($P$ value) less than 0.005 for all the variables. Table 2 lists mean, coefficient, and $P$ value for each cellular measurement. Thus, the tumor cells and their nuclei are nearly two times larger in FL-HCC as compared to HCC, and the nucleolus is nearly 3-fold larger, which is highly significant.

**Discussion**

Fibrolamellar carcinoma (FL-HCC) is an uncommon liver malignant neoplasm. Typically patients with FL-HCC are younger and show normal serum AFP levels as compared to conventional HCC. In addition, they do not have any pre-existing liver disease as compared to HCC patients who usually have background cirrhosis. Thus, in our study too the mean age of FL-HCC patients was 19 years and all presented with a large liver space occupying lesion in a non-cirrhotic liver on imaging studies. Histologically, FL-HCC is characterized by well-differentiated malignant hepatic cells with deeply eosinophilic and granular cytoplasm due to the presence of numerous mitochondria, and by the presence of thick, fibrous lamellae throughout the tumor. The case for distinguishing FL-HCC from HCC is supported by reports of better prognosis with FL-HCC compared to HCC. The cytomorphology of FL-HCC from liver fine needle aspiration has been described previously in a few case reports. There are also a few reports of cytological diagnosis of FL-HCC in metastatic sites and role of EUS-FNA has been highlighted. There is only a single case series of 6 cases, which only describes the cytology along with morphometry. Presence of large polygonal cells with abundant eosinophilic cytoplasm having large vesicular nuclei, and large nucleoli are characteristic and in conjunction with the lamellar fibrosis, which also can be appreciated in cytology smears are the defining features of FL-HCC. Cytoplasmic “pale bodies” were observed in all cases and were numerous in 1 cases; these have been reported to occur in 50% cases on histology. The exact composition of pale bodies is unclear, but they are immunoreactive for fibrinogen.

The role of immunocytochemistry in the distinction of FL-HCC from its conventional counterpart is described for the first time.
time in this case series. Review of the literature pertaining to immunohistochemical profile of FL-HCC on histopathology of resected specimen revealed that HepPar1, CK7, EMA, and CD68 are constantly expressed in the bulk of tumors; CK19 is usually negative.\(^{[21]}\) CK7 is a marker of bile ductular differentiation and it is expressed in FL-HCC allowing its distinction from HCC.\(^{[20]}\) A recent study found that FL-HCCs are uniformly positive for CD68.\(^{[22]}\) Hence, we hypothesized that FL-HCC and HCC can be distinguished by a minimum panel comprising of CD68 and CK7 and applied it in four cases with available cell blocks. Although CD68 was positive in FL-HCC with canalicular and cytoplasmic positivity, it also showed similar canalicular positivity in HCC limiting its utility in their distinction. On the other hand, CK7 proved to be discriminatory as all cases of FL-HCC showed uniform, diffuse, and strong cytoplasmic and membranous positivity, whereas conventional HCC showed focal and weak expression [Figure 2].

FL-HCCs are locally aggressive and have a high frequency of distant metastases. Based on imaging findings, 42% of FLCs extend outside the liver.\(^{[23]}\) Overall, lymph node and peritoneal metastases are more common in FL-HCC than in typical hepatocellular carcinoma.\(^{[17]}\) Lymph node metastasis was seen in two cases in the present series. The favorable prognosis in FL-HCC has been attributed to a high operability rate due to its occurrence in a non-cirrhotic liver as compared to HCC.\(^{[17,18,24]}\) If resection is complete, prognosis is excellent, but if not clinical behavior of FL-HCC is similar to that of HCC.\(^{[25]}\) Follow-up surgery and histopathology confirmation was available in only two of the six cases in this series.

Morphometry helps in quantification of what is perceived qualitatively. Thus in FL-HCC, the malignant hepatocytes were nearly 2 times larger than the cells of HCC and 3 times larger than a ‘normal’ hepatocyte. Not only the cells, but also the nuclei and nucleoli of FL-HCC are larger in FL-HCC as compared to HCC. However, the nucleo-cytoplasmic ratio tends to be lower in FL-HCC. Similar findings are also documented in the previous report on 6 cases by Perez-Guillermo et al. with cells of FL-HCC showing larger cell size as well as larger nuclear and nucleolar dimensions along with relatively lower N:C ratio in comparison to cells of HCC.\(^{[16]}\)

**CONCLUSION**

Fibrolamellar-HCC can be distinguished from conventional HCC based on the characteristic cytomorphological features along with CK7 immunocytochemistry on cell blocks.

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**Conflicts of interest**

There are no conflicts of interest.

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