

Hepatocellular carcinoma

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ABSTRACT

Objective: To establish the most common pathological criteria to diagnose hepatocellular carcinoma, and to identify the high-risk patients for further investigation in order to detect hepatocellular carcinoma in its early stages.

Methods: A retrospective study was carried out at King Khalid National Guard Hospital including 60 cases diagnosed as hepatocellular carcinoma cytologically and histopathologically. Further investigations were performed by special staining and immunohistochemical staining on 42 blocks including Periodic acid-schiff, PAS-D, Reticulin, Iron and Alpha Fetoprotein, hepatitis B surface antigen, hepatitis B core antigen and p53 antibodies.

Results: It was found that pleomorphism, followed by presence of prominent nucleoli and nuclear pseudoinclusion were the most frequent finding in

hepatocellular carcinoma. While considering other studies, reticulin framework, glycogen and iron content of the hepatocellular carcinoma were markedly diminished as compared to non-malignant liver tissue. Antibodies against tumor suppressor gene was applied on paraffin section (p53), it was positive in 52% of cases and 53% of them were having anti hepatitis B surface antigen positivity, detected in their serum and 23% were having hepatitis C antibodies positive.

Conclusion: Hepatocellular carcinoma is a common malignancy that can be detected by certain defined pathological parameters and should be suspected in high-risk patient.

Keywords: Hepatocellular carcinoma, p53, screening program, chronic active hepatitis.

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The prevalence of Hepatocellular carcinoma (HCC) is highest in Saudi males, according to the recent statistics published by Saudi National Cancer Registry.¹ Prior to the development of this registry, HCC was ranked fourth in males, seen in the highest referral institution, preceded by Non Hodgkin's Lymphoma, esophageal and lung cancer.^{2,3} The most common age at presentation is the sixth decade. The highest frequency of HCC was noticed in Gizan and Riyadh areas with male to female ratio of 4:1. Hepatitis B virus was positive in 65% of cases.⁴ Worldwide, hepatocellular carcinoma shows largest significant geographical variation in incidence. The highest incidence rate of 20-150 new cases/100,000 population per year, was seen in the East, West and the Central black Africans, Southeast China, Taiwan, Korea, Vietnam, Hongkong and Singapore. An

intermediate incidence rate, with number of new cases ranging between 5-20 new cases/100,000 population per year that was seen in Southeast Asia, South Africa, Japan, Middle East, India, Pakistan, Central America, Alaska, South and East Europe. The lowest incidence of new cases was less than 5/100,000 population per year, and was seen in North America, South America, Northwest, Central Europe, Australia, North Africa, South Africa and Central Asia.⁵ The most common predisposing factor of HCC in Western countries is liver cirrhosis, however in developing countries, hepatitis B virus infection is the main predisposing factor. In a study published from Riyadh, Saudi Arabia, hepatitis surface antigen (HBsAg) was found in the serum of 60% of cases.⁶ Another similar study from Gizan area showed positive HbsAg in 65% of cases.⁴ A third study of

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HCC shows HBsAg in 58% of cases as well as high titer of schistosomal antibodies in 36% of their cases.⁷ The morphological features of HCC were discussed in details in hepatopathology and cytopathology.^{5,8-10} The utilization of special stains such as reticulin, PAS and iron are well discussed in previous articles.¹¹⁻¹⁵ This study was directed to emphasize upon the usage of a set of criteria that can be used both in histology and cytology in order to diagnose HCC more accurately and to use special stains and immunohistochemical stains to confirm the diagnosis.¹⁶

Methods. Retrospective study including total of 60 cases of liver biopsies diagnosed as HCC were carried out at King Khalid National Guard Hospital (KKNHG) between 1985-1997. All the slides were re-evaluated. The material from 40 cases were liver biopsies, 20 cases had fine needle aspiration cytology (FNA), only 4 cases had both fine needle aspiration and biopsy. The following features were looked for, (1) Cell morphology, nuclear shape, pleomorphism, nuclear pseudoinclusions, presence of nucleoli, amount of cytoplasm, cytoplasmic vacuoles, presence of multinucleated giant cells, bile formation, number of normal and abnormal mitosis; (2) Architecture pattern, trabecular pattern (2 cells in a row), sinusoidal pattern, the later show hepatocytes in rows or groups surrounded by endothelial cells, and glandular pattern; (3) Special Histochemical stains, PAS, PAS-D, reticulin and iron were performed on these sections. Immunohistochemical staining with monoclonal antibodies directed against (tumor suppressor gene) p53 (Daco), p53 was monoclonal mouse antibodies directed against human p53 protein. Alphafetoprotein (AFP) (Daco), HbsAg (Daco) were also carried out. The special staining and immunohistochemistry were performed on paraffin blocks of 42 cases; (4) The adjacent non-neoplastic liver tissues were evaluated for cirrhosis, chronic active hepatitis (CAH), chronic persistent hepatitis (CPH), fibrosis and fatty changes. The cytological materials were also assessed by the same criteria used in histological sections (morphology and architecture) as well as cellularity.¹⁶ The cytology slides were stained with Romanovsky stain (diff quick), pap stain and H&E. The available serology of the cases tested for Hepatitis B & C were retrieved from the archive and analyzed.

Results. The cell morphology in both biopsy and cytology were similar in all cases when they were examined. The tumor cells exhibited pleomorphic round-oval nuclei with prominent central nucleoli. Intranuclear pseudoinclusion was found in 100% of cases in the cells of the biopsy specimens and in 89% of cytology specimens. Moderate amount of cytoplasm was found in all the cells, and cytoplasmic vacuoles were seen in 65% of cases.

Intracytoplasmic bile was present only in 27% of cases. Multinucleated giant cells were noted in 46% of biopsy specimen and in 80% of cytology specimens with some abnormal form seen only in 16% of both specimens. Well-differentiated HCC represented 58% of the cases, while moderately differentiated HCC was 33% and poorly differentiated HCC represented 8%. Ninety percent of fine needle aspiration were cellular. The histochemical staining showed diminished glycogen stores in 48% of the cases of HCC as compared to normal liver glycogen. Reticulin fibers were markedly diminished around the cell clusters in 97% of HCC cases as compared to normal pattern. In addition, iron was also markedly diminished in 92% of the cases when compared to normal. Immunohistochemistry showed alphafetoprotein positively in 50% of cases. P53 antibodies were positive in 52% of cases. This positivity based on nuclear staining is more positive in 50% or more of the tumor cells present in the sections examined. All cytoplasmic staining were disregarded. Antibodies for HbsAg were positive in 40.5% of the cases while HBcAg was positive in 45% of the cases. 40% of these cases have both HbsAg and p53 positivity. Serology results were analyzed at and it was found that 18 out of 34 cases were positive for hepatitis B surface antigen (53% HbsAg), 4 out of 22 cases for hepatitis B core antigen (18% HBcAg) and 8 out of 35 cases for hepatitis C antibodies (23%).

Discussion. Hepatocellular carcinoma is the most common tumor seen in Saudi males. The list of differential diagnosis of HCC is long, and contains benign and malignant lesions.^{11,17} The benign lesions are focal nodular hyperplasia, liver cell adenoma and macronegneranodules of cirrhotic liver. The malignant tumors include metastatic carcinoma, lymphoma and malignant melanoma. FNA procedure is becoming a very popular method as first diagnostic step among invasive techniques to reach the diagnosis.⁹ It is a challenging situation for pathologist to make an accurate diagnosis from small hepatic nodules. Therefore, they are required to identify a specific well-defined criterion to reach the diagnosis on small biopsy and cytology aspirate. The criteria listed before was applied in this study to recognize the nature of hepatic masses. This study in agreement with others showed that the cellular morphological changes and the architectural pattern are very helpful in identifying HCC.^{5,9,11,15,18,19} The presence of bile is very specific for HCC, yet it is seen in only a small fraction of cases i.e. 27%. The sinusoidal pattern is the most common pattern seen in HCC, followed by the trabecular and pseudoglandular pattern, however, according to WHO the presence of these patterns qualify for the diagnosis of HCC.¹⁹ This study showed the sinusoidal pattern was present in half of cytology and

in the majority of the biopsy specimen, and the pseudoglandular pattern was seen in only few cases. Ferel study,¹¹ and others^{10,13,14} elaborated on the utilization of reticulin to differentiate between a small focus of HCC from atypical macroregenerative nodule. Irregular thickened plates are present in the latter, while there is loss of reticulin fibers in 98% of HCC cases. Furthermore glycogen content and iron content is reduced in HCC which is inconsistent with Ferel's findings. Mutation of p53 has been implicated in the carcinogenesis of many tumors including HCC. The presence of over expression of p53 oncoprotein is identified in 52% of the cases in this study, however 40% have both p53 expression and HbsAg by immunohistochemical staining which is slightly lower expression than the study of Skopelitou et al²⁰ where p53 was identified in 47% of their cases who had positive serology for HbsAg and HCV. Absence of p53 oncoprotein expression and negative HbsAg was seen in 21% of the cases and this might implicate a different mechanism of carcinogenesis other than mutation of tumor suppressor gene p53. In our part of the world, environmental factors such as schistosomiasis may be an important factor in carcinogenesis. Nouh et al⁷ in their study of 50 cases of HCC found a significant titre of schistosomal antibodies in 36% of cases. Their results for positive hepatitis surface antigen serology were 58%, which is comparable to our study i.e. 53%. On the other hand Al Krawi²¹ identified HCV in 30% of their HCC cases, which is comparable with 23% in this study. These findings emphasize the importance of HCV and HBV infection in the causal relationship to HCC.²² The relationship between cirrhosis and HCC is well documented in the literature.^{5,12,22,23} In fact, a well-established cirrhosis was noticed in almost one third of the cases in the adjacent non-malignant liver in this study. In addition, portal fibrosis was seen in two third of the case. Furthermore chronic active hepatitis was a significant feature seen in the non-malignant liver in 41% of cases. The later finding emphasizes the importance of considering patient with CAH as a high-risk group, and should be screened for HCC.^{18,24}

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