Bahrain Medical Bulletin, Vol. 29, No. 2, June 2007

An Update on the Pathogenesis and Pathology of Hepatocellular Carcinoma

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Primary malignant neoplasms of the liver arise from hepatocytes, intrahepatic bile ducts, blood vessels and endothelial cells (*Box 1*).

Hepatocellular carcinoma (HCC) is a malignant neoplasm of hepatocytes and constitutes more than 80% of primary malignant liver neoplasms. HCC is the preferred terminology and terms like "hepatoma" and "liver cancer" should be avoided because they are not precise.

Hepatocellular carcinoma (HCC) is second only to carcinoma of the pancreas in being the most lethal form of human cancer[1]. Almost all patients die within 6-7 months after the diagnosis. This is especially true in areas of high endemicity. This dismal prognosis is due to lack of reliable biomarkers that permit early diagnosis, resistance of the tumour to chemotherapy and the underlying diffuse liver disease that limits the use of chemotherapeutic agents in medical management. Other than primary prevention, one hope of changing the prognosis is early diagnosis (See below).

HCC is the fifth most common internal malignancy world wide and the commonest internal malignancy in men below the age of 45 years in Sub-Saharan Africa. There is significant geographical variation. The tumour is common in China, Southeast-Asia and Sub-Saharan Africa where the prevalence is estimated to be 100/100,000 population as compared to 3/100,000 in Europe and U.S.A.

Box 1: Primary malignant neoplasms of liver

- Hepatocellular carcinoma
- Hepatoblastoma
- Cholangiocarcinoma
- Bile duct cystadenocarcinoma
- Haemangiosarcoma
- Epitheloid haemangioendothelioma

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Hepatocellular carcinoma is asymptomatic for most of its natural history. Symptoms attributable to HCC develop in the late stages of the disease. Cirrhotic patients who develop HCC present with accelerated liver decompensation manifested as deepening jaundice or ascites. Worsening portal hypertension might indicate extension of the tumour into the portal vein. Extension into the hepatic vein leads to Budd-Chiari Syndrome. Haemoperitoneum might result from rupture of the neoplasm into the peritoneum. Rarely, HCC mimics liver abscess and the patients present with fever, leucocytosis and tender liver mass.

Pathology

There are several gross classifications and descriptions of hepatocellular carcinoma. Most of these classifications are refinements of Eggel's classification of 1910. Eggels classified the gross appearances of HCC into three categories: massive, nodular and diffuse[2]. Massive designates a single large mass with or without satellite nodules; nodular is composed of multiple discrete nodules; and diffuse shows numerous nodules covering the entire liver resembling cirrhosis.

Microscopic diagnosis of HCC hinges on the identification of cellular and growth patterns indicative of liver cell differentiation. Large cells with granular cytoplasm, presence of bile canaliculi, evidence of bile secretion and trabecular growth pattern are helpful microscopic diagnostic features.

There are varieties of cellular cytoplasmic inclusions, which are characteristics but not diagnostic of HCC. Mallory hyaline, fibrinogen inclusions, PAS positive α-1-antitrypsin globules, Dubin-Johnson like pigment and glycogen are examples.

Hepatocellular carcinoma is classified into several histological types. These include trabecular, pseudo-acinar and compact growth patterns. Tumours with stromal fibrosis are designated as schirrus and those with dilated stromal blood vessels and vascular lakes resembling peliosis hepatis are known as peloid HCC. Anaplastic HCC can be so undifferentiated that it has little or no resemblance to liver cells. Unfortunately, none of these classes has a bearing on prognosis or response to treatment except fibrolamellar carcinoma which will be considered separately.

Because of the variable growth pattern of HCC, it sometimes presents a diagnostic challenge. The pathologist needs to differentiate HCC from other primary liver neoplasms especially liver cell adenoma, dysplastic nodules and cholangiocarcinoma. Metastatic neoplasms, being the commonest liver malignancy can sometimes pose a diagnostic challenge to the pathologist.

Fibrolamellar carcinoma

This is a true variant of HCC. First described by Edmonson in the Armed Forces Institute of Pathology fascicle of tumours of the liver of 1958[3]. The same group reported their experience in 1980 and coined the name fibrolamellar carcinoma[4]. In contrast to classical HCC, this tumour occurs in a younger age group (5-35 yrs),

affects males and females equally and is not associated with the HBV, HCV, Alfatoxin or cirrhosis. α-Fetoprotein is raised in only 10% of the patients.

Macroscopically, 75% of fibrolamellar carcinomas affect the left lobe. More than half the tumours present as a single mass with central scar resembling focal nodular hyperplasia grossly and radiologically. The non-neoplastic liver is usually normal.

Microscopically, the tumour cells grow in sheets of large cells with eosinophilic granular cytoplasm. Bile, glycogen, fat and α-1-antitrypsin globules are sometimes seen in the cytoplasm. The stroma shows sheets of fibrous tissue with occasional smooth muscle or thick walled blood vessels. Occasionally bile ducts are seen within the neoplasm. Inflammatory cells including histiocytic granulomas are rarely noted[5].

The microscopic differential diagnosis includes focal nodular hyperplasia and classical HCC and other malignant liver neoplasms. The central scar, fibrous septa, thick walled blood vessels and bile ducts are also features of focal nodular hyperplasia. Some are of the opinion that fibrolamellar carcinoma is malignant counterpart of focal nodular hyperplasia. Fibrolamellar carcinoma is a resectable tumour in around 60% of cases. Its five year survival rate is around 56%.

Diagnosis of HCC

α-Fetoprotein is synthesized by foetal hepatocytes and yolk sac cells. It is raised in germ cell tumours of the testis and ovary and in 80% of HCC. The normal range of serum α-fetoprotein is 10-20 ng/ml. Levels above 400 ng/ml are diagnostic of HCC (in absence of a gonadal germ cell tumour). Serum α-fetoprotein is used in the early diagnosis of HCC (see below). α-Fetoprotein can also be demonstrated in the tissues by immunoperoxidase technique.

Several antibodies are available that help differentiate HCC from other neoplasms. Hepatocyte paraffin 1 (Hep Par1) or Hepatocyte specific antigen (HSA) has granular cytoplasmic positivity in normal liver cells and most HCC. Although it is positive in a minority of gastric carcinomas, it is a sensitive marker for HCC. Monoclonal antibody to carcinoembryonic antigen (mCEA) is positive in a wide range of carcinomas but negative in HCC. Polyclonal antibodies to carcinoembryonic antigen (pCEA) are positive in HCC and other carcinomas, but it produces distinctive diagnostic canalicular pattern in HCC. The staining characteristic of Cytokeratins 7, 20, 8, and 19 are helpful in sorting out metastatic adenocarcinoma, cholangiocarcinoma and HCC.

Etiology and Pathogenesis of HCC

Several factors contribute to the pathogenesis of HCC. (*Box 2*). HBV, HCV and Aflatoxin account for more than 80% of HCC worldwide. These agents alter the function of groups of genes involved in the control of cell growth, apoptosis and DNA repair. The chronic hepatic inflammatory response induced by HCV, HBV and aflatoxin induces cycles of liver cell destruction and regeneration and create the microenvironment for the propagation of these genetic changes thus contributing to the multistep causation of HCC.

Box 2: *Causes of Hepatocellular Carcinoma*

HBV

HCV

Aflatoxin

Alcohol

Cirrhosis

Metabolic diseases:

- Hereditary haemochromatosis
- Alpha-1 antitrypsin deficiency
- Tyrosinemia
- Glycogen storage disease
- Porphyria cutanea tarda

Age and Gender

Hepatocellular carcinoma is more common in males than females. This is independent of the fact that men are more prone to HBV infection than women. The ratio for males and females ranges from 2:1 to 5:1 in HCC. The corresponding ratio for HCV infection is 1.2:1. The cause of this male preponderance is not known.

Most of the etiological factors of HCC especially HCV and HBV infection take about 20-30 years to result in HCC. In developed countries, the average age of patients with HCC is 65 years. In endemic areas, there is a bimodal distribution with peaks at 45 and 65 years. In Mozambique, HCC is the commonest malignancy in men below the age of 40 years.

HBV

Case control studies have shown that chronic HBV infection increases the risk of HCC 100 times. Reports from Taiwan show evidence that mass vaccination against HBV infection reduces the incidence of HCC. Two billion individuals are infected by HBV worldwide. HBV causes about 320,000 deaths annually; up to 50% of these deaths are attributable to HCC. It is estimated that HBV accounts for over 70% of HCC worldwide.

HBV is a member of the DNA hepatotropic viruses that belong to the Hepadnaviridae family[1]. The mammalian hepadnaviruses, also known as ortho-hepadnaviradae, include the woodchuck hepatitis virus (WHV) and the ground squirrel hepatitis virus (GSHV). Avi-hepadnaviruses are restricted to birds and infect grey herons, geese and Pekin ducks. The prototype of this group is duck hepatitis B virus (DHBV).

Ortho-hepadnaviruses infect their respective host and are known to produce persistent chronic infection and cirrhosis. There is high incidence of HCC in mammals approaching 100% in woodchucks infected by WHV. This has been an excellent animal model for investigating HBV related HCC. Avi-hepadnaviruses on the other hand do not induce HCC in birds[6].

HBV is a partially double stranded DNA virus. Its genome has four long open reading frames (ORF). The surface and pre-surface region (pre S-S) encodes three surface antigens the most important of which is HBsAg. M protein encoded by preS2 has no known function. L protein which binds the virus to the host cell is encoded for by preS1. Pre-core and core regions (pre C-C) encode hepatitis B core and e antigens (HBcAg, HBeAg). The P coding region encodes polymerase enzyme responsible for DNA synthesis. The X ORF encodes viral X protein (HBX). The X protein is required for viral replication and spread.

The X gene and protein is present in ortho-hepadnaviruses and absent from avi-hepadnaviruses. Since avi-hepadnaviruses are not associated with HCC in birds, the X gene and its 154 amino acid protein are thought to play important roles in the maintenance of infection and the causation of HBV-associated HCC.

HBV virus contributes to the pathogenesis of HCC through several mechanisms. HBV integrates part of its DNA genome at several locations into or close to cellular genes that control cell growth and apoptosis. The cellular genes affected include telomerase reverse transcriptase (TERT) and platelet-derived-growth factor receptor-β (PDGFRβ)[1,7].

HBX binds p53, inactivates it and as such interferes with apoptosis and DNA-repair check points. p53 is known to suppress α-fetoprotein gene. p53 and HBX interaction relieves the suppression of α-fetoprotein gene and leads to increased α-fetoprotein synthesis in 80% of HCC. HBX also activates growth control genes such as SRC, tyrosine kinases, Ras, Mitogen-activated-protein kinase (MAPK) and others.

Hepatitis C Virus (HCV)

HCV is an RNA virus of the flaviviridae family. Its genome encodes NS2, NS3, NS4A, NS5A non-structural proteins in addition to E_1 and E_2 envelope proteins. About 75% of individuals infected by HCV develop chronic infection as compared to 10% of HBV infected individuals. Chronicity of HCV infection is related to a number of factors that contribute to evasion of host immune response by the virus. One factor is the frequent mutations in the viral genome creating several quasi-species. HCV core proteins are known to impair dendritic cell function and consequently suppress T cell activation. HCV proteins NS3, NS4A have protease activity that cleaves and inactivates components of the immune response.

HCV core proteins also interact and activate MAPK signaling pathway. NS5A combines with p53, sequester it to the nuclear membrane, and affects its activity[1,7].

Aflatoxins

In the 1960s, an outbreak of acute poisoning characterized by fatal haemorrhagic liver necrosis affected poultry and was traced to fungus contaminated feeds. Outbreaks of HCC in rainbow trout fisheries were also traced to the same cause. Later a number of mycotoxins were isolated, the most significant being aflatoxins produced by Aspergillus flavus. They were designated B_1, B_2, and G_1, G_2 due to their blue and yellow-green autoflourscence. Aflatoxin B_1 is the most potent. In large doses, it induces acute hepatotoxicity in animals. Adult humans are tolerant to high doses of Aflatoxin B_1 and acute toxicity is rarely reported. Most of the cases reported occur in children.

In small doses over a long time, Alfatoxin B_1 is one of the most potent hepatocarcinogens. Its hepatocarcinogenic effect is more pronounced in males. Experimentally Alfatoxin B_1 induced HCC in almost all animals tested: rats, marmosets, ferrets, tree shrews, ducks and rainbow trouts. The mouse is resistant to Aflatoxin induced HCC.

The International Cancer Institute identified Aflatoxin B_1 as a class I carcinogen and recommended that its concentration in grain should be less than 20 ppb in USA and Europe[8]. Underdeveloped countries do not have the means and cannot afford the cost of implementing these regulations. As such, Aflatoxin remains an important cause of HCC in China and Sub-Saharan Africa, especially when coupled with HBV infection. There is evidence of synergism between Aflatoxin B_1 and HBV in the pathogenesis of HCC. One study indicates that patients infected with HBV are seven times more likely to develop HCC. Those exposed to Aflatoxin (as measured by urinary Aflatoxin metabolites) are three times more likely to develop HCC. If both HBV infection and aflatoxin exposure are present the risk of developing HCC rises to 60 times.

Aflatoxin B_1 is metabolized by Cytochrome p450 into exo-8, 9 epoxide[9]. Aflatoxin B_1 epoxide forms adducts with DNA predisposing to DNA mutations. The favourite site for Aflatoxin induced adducts is a hotspot in the p53 gene. This leads to GC-TA transversion of the third position of codone 249 of p53 gene resulting in Arg $\rightarrow$ Ser alteration of p53 protein. This mutation is detected in 50% of HCC from areas where Aflatoxin contamination is high, and in only 1% of HCC from USA, Europe, Japan and Australia.

P53 is a tumour suppressor gene. It acts as transcription activator that regulates the cell cycle. It also plays a role in apoptosis and DNA repair. P53 inactivation or mutations are a common pathway for the different causative factors of HCC.

Liver Cell dysplasia

In most carcinomas that arise from epithelial surfaces, pathologists recognize changes in the light microscopic appearances of cells that predate the appearance of carcinoma by up to 5 years. These changes are called dysplasia. Solid glandular organs like the liver are no exception. Liver cell dysplasia was first described by Anthony in 1973. Dysplasia was seen in 1% of normal livers, 6.9% of cirrhotic livers and 64.5% in cirrhosis with HCC[10].

Since then the classification of liver cell dysplasia with or without nodule formation became complicated. One attempt at a unified classification scheme for dysplastic foci and dysplastic nodules was published in 1995 by International working party[11]. Dysplastic nodules are precancerous. They are classified into high grade and low grade dysplastic nodules. There is evidence that high grade dysplastic nodules progress to HCC. Dysplastic nodules are usually less than 10 mm and nodules larger than 15 mm are usually malignant.

Early Diagnosis of HCC

Early HCC is asymptomatic. Thirty percent of patients undergoing liver transplantation for HCV associated cirrhosis have silent HCC. Early diagnosis of HCC depends on screening of patients at risk. In developed countries, 90-95% of patients with HCC have cirrhosis with varying etiology. The British Society for Gastroenterology guidelines are reported by Ryder[12]. High risk groups include males and females with cirrhosis due to HBV, HCV and haemochomatosis; and males with alcoholic cirrhosis who abstain from alcohol.

Patients are investigated initially by ultrasound examination and serum α-fetoprotein levels. Patients with nodules ≥ 20 mm and serum α-fetoprotein level ≥ 400 ng/ml are diagnosed as HCC and no confirmation by liver biopsy is required. Such patients are further evaluated by spiral computerized tomography (spiral CT) and MR1 before given medical or surgical management.

Two-thirds of patients with HCC < 40 mms have α-fetoprotein < 200 ng/ml, which is below the diagnostic range. Since the average doubling time for HCC is 6 months, such patients are evaluated at 6-monthly intervals. A rising level of serum α-fetoprotein or increasing nodule size is taken as diagnostic of HCC.

Several protocols for grading and staging of early HCC are used[13]. The most popular are those developed by Cancer of the Liver Italian Programme (CLIP) and Barcelona Clinic Liver Cancer (BCLC). Factors considered significant in the prognosis of early HCC are tumour size, number of nodules, serum albumin and serum bilirubin.

Early diagnosis and management has markedly increased the 5 year survival rate of patients with HCC. Vaccination against HBV has already reduced the incidence of HCC in Taiwan. There is hope that mass vaccination might eradicate HBV infection worldwide and thus eliminate one of the major causes of chronic liver disease and HCC.

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