Hepatocellular Carcinoma: Clinical Features, Evaluation and Treatment

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. It accounts for 80 to 90% of all primaiy tumors of the liver, but is seen infrequently in the United States and accounts for less than 2% of all malignancies. Similarly, a low incidence is reported in Britain, Canada and Australia. However, in certain parts of Africa and Asia, HCC is the most common malignant tumor. Incidence in these high risk areas varies from 34/100,000 men in Singapore to 100/100,000 men in Mozambique and Taiwan[1]. HCC is seen more frequently in men greater than 30 years of age. In high incidence areas, the male to female ratio is 5:1, whereas in low incidence areas the ratio is reduced to 2:11. In Madras (India), age adjusted incidence of HCC in males is 2.1/100,000 and in females 0.7/100,000[1].

Etiologic Risk Factors

The exact etiology of HCC is unknown. However various genetic and environmental factors have been implicated. Cirrhosisispresentin6Oto 90%ofHCCpatients inAsia and Africa[2]. In most of these cases, cirrhosis is post-viral. Cirrhosis caused by hemochromatosis or alcoholism has also been associated with high incidence of HCC.

Chronic hepatitis B virus (HBV) infection increases the chances of developing HCC[4]. Amongst patients with HBV infection, incidence is increased in those with active hepatitis or persistent antigenemia. This association has been well documented in a number of studies[3,4]. HBV DNA has been found integrated withinthe chromosomal DNA oftumorcells. Using appropriate restriction endonuclease diagestion and southern blot analysis, this genomic integration has been demonstrated in almost three- fourths of patients with HCC[5]. Once integrated, HBV DNA may be extensively rearranged and is possibly associated with host chrornosornal damage. The integrated HBV DNA is functional. RNA transcripts can be translated into protein products, HBs Ag, which is detectable by immuno-histochemical staining in approximately one third of HCC patients.

Several mechanisms have beenpostulated to explain the putative role played by the hepatitis B virus in the causation of HCC. These include the possibilities that HBV may contain transforming genes that produce oncogemc products, or virus-induced rearrangement of cellular DNA may alter genes controlling cell growth, or integrated viral DNA may contain promoter and enhancer segments whichmight inappropriately activate cellular oncogenes.

More recently, Hepatitis C virus (HCV) has been implicated in causing HCC. Nearly 29% patients with HCC in South Africa are anti-HCV positive[6]. In Europe, 39 to 76% of the patients with HCC are positive for anti-HCV antibodies[7-10]. In a prospective study of 917 patients with chronic liver disease from Osaka, Japan, it was observed that risk of liver cancer increased almost four-folds in patients with anti-HCV antibodies[11].

In an Italian case control study, 71% patients with HCC were anti-HCV positive as compared to 5% controls who had non-hepatic chronic disease. In the same study, it was found that 74% patients with HCC who also had cirrhosis were anti-HCV positive as compared to 62% patients with cirrhosis alone. This shows that HCV plays a role in developing cirrhosis and also increases the risk of developing

HCC[12].

HBV and HCV infections and integrations are the initiating events in hepatic carcinogenesis. Any one or a more of a number of causes that result in active regeneration of hepatocytes may represent the promotional events in causation of HCC.

Aspergillus Flavus, a fungus found in peanuts is implicated in etiology ofHCC in Africa and Asia[13]. In Taiwan, DNA adducts ofAflatoxinB 1 in smeared tumor tissue from 50 patients with HCC were studied. Indirect inununofluorescence assay combined with densitornetry was utilized to quantitate Aflatoxin DNA adducts. Monoclonal antibody 6A10 against Aflatoxin B 1 adducts was used for detection. Nearly 70% HCC patients had detectable levels of Aflatoxin DNA adducts (>$1/10^6$ nucleotides). The results suggest that Aflatoxin B1 may be involved in the pathogenesis of HCC in Taiwan[14]. Similar studies are required in other patient populations. Alcoholism is also implicated in the etiology of HCC. but the association is less strong. Alcohol gives rise to alcoholic cirrhosis, a chronic liver disease, which may predispose the patient to develop HCC. Most HCC occur in men suggesting hormonal involvement. It has been observed that some hepatic tumor cells express surface estrogen and androgen receptors. Inaprospective study from Taiwan, serum samples of 9691 male adults were collected and frozen. With a mean follow-up of 4.6 years, testosterone levels in the stored sera were measured for 35 cases of newly diagnosed HCC, 63 hepatitis BsAg negative and 77 Hepatitis BsAg positive matched controls. Elevated serum testosterone levels correlated with an increased risk of HCC. This association remained significant even after the adjustment for otherHCC risk factors[15]. Drugs that block the testosterone recepiors are being evaluated as therapeutic modalities in the treatment of HCC[16].

Clinical Features

HCC occurs most commonly in middle aged to elderly men. Patients often present with constitutional symptoms like anorexia and weight loss. Many patients complain of abdominal pain and discomfort which may be acute if there is hemorrhage into the tumor. On examination, there may be a palpable irregular mass arising from the liver. Since HCC is a very vascular tumor, a hepatic bruit may be heard. If clinical signs and symptoms directly referable to HCC have developed, the prognosis is grave. The differences in clinical features of HCC in high and low incidence areas are given in Table I.

Table I. Clinical features of hepatocellular carcinoma in high and low incidence areas.

Clinical characteristic	High incidence	Low incidence
Geographic area	Asia/Africa	North America/Europe
Race	Asian/Black	Mostly white
Median age	A:40-50 years B:20-30 years	50-60 years
Duration of symptoms	Usually short especially in young blacks	Can be indolent
Abdominal pain or discomfort	70-90%	50-70%
Anorexia and weight loss	Common	Common
Haemorrhage: secondary to rupture of tumor	10-20%	<10%
Cirrhosis	60-80%	60-80%
Cirrhosis evolving to HCC	50%	5-10%
Type of cirrhosis	Mostly macro-nodular	Mostly micro-nodular
Etiology of cirrhosis	HBV>HCV	HCV >HBV
HCC associated with HBV	80%	30-50%
Hepatitis B antigen present	70-90%	15-40%
Exposure to aflatoxin	High	Unlikely
α-Fetoprotein 400mg/ml	70-85%	30-65%

Diagnosis

Patients at the time of presentation, having clinical signs and symptoms referable to HCC carry a dire prognosis. Recent emphasis is on detection of small/asymptomatic cancers at potentially curable stage. Screening is possible in high risk populations, such as patients with cirrhosis and chronic hepatitis C virus, or hepatitis B virus carriers. Sensitive immunoassays can detect increase in serum alpha fetoprotein (AFP) or des-garnma-carboxyprothrombin in some patients with small and asyniptornatic HCC[17,18]. This in combination with liver ultrasound, increases the diagnostic yield in screening studies.

Tumour markers

Aipha-fetoprotein: In healthy adults, Serum AFP concentrations are usually less than 20 uVml but in patients with HCC it may increase to >400 ug/ml[19]. . AFP levels are high in 70 to 80% of the patients with HCC[20]. Levels are also increased in metastatic disease, endodermal tumors, pregnancy etc. In an Alaskan study, AFP was observed to be a sensitive marker specially in men, with a positive predictive value of 43%[21]. It is also useful for monitoring recurrence. Serum AFP levels correlate closely with tumor size[22]. Although most symptomatic HCC are associated with AFP levels >1000 ug/ml, this is not

true for small HCC. About, two thirds of patients with small HCC will have an AFP level of less than 200 ug/ml. A new assay of AFP using monoclonal antibody may enable the clinicians to distinguish benign from malignant liver disease[23].

Des-gamma-Carboxyprothrombin: An abnormal prothrombin which is found in the serum of patients with HCC. Upto 75-90% of patients with HCC may have this marker detectable in their serum[24]. Levels are undetectable in the normal subjects. In a study of biopsy proven HCC, the mean level of abnormal prothrombin was 900 ug/ml. It was detectable in 91% patients suggesting it to be a useful tumor marker for HCC[24]. A number of othermethods have also been used to diagnose and screen forHCC. These include abnormal variants of alkaline phosphatase, gamma-glutamy I transpeptidase isoenzvmes,isoferritins, carcinoembryonic antigen and specific tumor antigens detected by the monoclonal antibodies[25-27]. None has yet proven better than AFP as a screening test.

Imaging modalities

Any hepatic mass in a patient with chronic hepatitis or cirrhosis must be considered malignant and differentiated from a benign lesion. A numberof imaging modalities are used to detect HCC. Ultrasonography is frequently used to screen high-risk populations and should be the first study done when HCC is suspected. Ultrasonography can detect tumors 1 cm in size or more[28]. It is inexpensive and an effective diagnostic and screening tool. For most tumors, it is probably as sensitive as any other imaging modality[29]. It has equal or increased sensitivity compared to radionucleide scans. It can also be used to guide the aspiration needle and to better define the anatomy. In one series of 51 patients, diagnosis of HCC with ultrasonography was confirmed in 72% cases[30]. Recently, a new method for the contrast enhancement of hepatic tumors using ultrasonography has been found to be useful. This uses ultrasound contrast enhancement with carbon dioxide microbubbles. This is currently one of the most sensitive methods for detecting small HCC[31]. Radionucleide-labelled colloid scan is also used for detection of HCC. It may be required if ultrasound is not helpful, if surgery is planned, or if uncertainty exists as to the extent of the tumor. Technetium 99m sulphur colloid is used for hepatic scintigraphy. This study is based on uptake of colloid by the hepatic reticuloendothelial system. It results in photopenic areas in the liver. In a study reported from Singapore, Tc-99 scan detected 94% patients with HCC, whereas detection with Gallium scan was 89%. Gallium scan is better in cases with lot of background activity due to cirrhosis. Computerized axial tomography (CAT) scan can detect and delineate the extent of hepatic tumors. It is relatively sensitive, non- invasive and can detect most tumors greater than 3 mm in size[32]. The role of CAT scan in detection of HCC is comparable to Magnetic Resonance Imaging. Coeliac axis angiography is sensitive and indispensable before surgery[33]. It gives information about the extent of the tumor and arteriovenous supply before surgical resection of the tumor. Lipoidol, an ether ester of poppy seed fatty acid oil combined with iodine, is a contrast medium which is, selectively retained intumorvessels and small tumor nodules. With this medium, millimeter sized tumors can be subsequently seen by CAT scan even several days afterwards[34]. By emulsifying an anti-tumor agent with lipoidol, the tumor can selectively be necrosed before surgery. Ultimate diagnosis of HCC requires histologic confirmation. Apercutaneousliverbiopsy orcytologic examination of fine needle aspirate should be done to differentiate the tumor from benign lesions. Positive histologic findings can be obtained in more than 90% of the patients with the above techniques. However, open liver biopsy increases detection rate to 98%[35]. Although histologic confirmation of HCC is necessary, possibility of dissemination of tumor along the biopsy needle track must be considered. Fine needle biopsy or aspiration of the tumor is therefore preferable. If histologic confirmation is not possible as in patients with cirrhosis and severe coagulopathy, a rising semmAFP, tumorvascularity on angiography and a focus of lipoidol retention are sufficient to establish the diagnosis of HCC.

Efforts are currently underway to evaluate precancerous and early cancerous lesions further by using

oncogene analysis, chrornosomal rearrangement and staining of the extracellular matrix antigens and Mallory bodies[36-38].

Staging criteria for hepatocellular carcinoma

A staging system based upon clinica lcharacteristics that recognizes the contribution of underlying liver disease has been developed by Okuda and colleagues[39] . This is presented in Table II.

Another pathologic tumor-node-metastasis (pTNM) staging system for hepatic tumors has been developed by the Union Intemationale Contra Le Cancer (UICC) and is outlined in Table III.

The natural history of Hepatocellular carcinoma

HCC can be unifocal, multifocal or infiltrative. A distinct clinicopathologic type is fibrolameller variety. This usually occurs in young adults, has no association with cirrhosis and carries a better prognosis.

Frequent presentation of HCC at an advanced stage has lead to two common misconceptions regarding the disease. Firstly, it is regarded as a rapidly growing tumour and secondly, that it is a universally fatal disease. In fact, HCC is slow growing and can be cured if detected and surgically resectedat an early stage[40,41]. HCC is relatively slow growing in comparison to other adenocarcinomas such as breast or colon cancer. Growth rate of this cancer has been documented in studies by Sheu and co-workers from Taiwan[42,43]. They performed serial ultrasound examinations on patients with HCC who, for various reasons, were followed up for prolonged time without therapy. Doubling time of the tumor ranged from 1 to 14 months, the median being four months. Furthermore, for early detection of an average growth rate HCC, screening need only be done once a year. This should detect most tumors at a stage when they are still potentially resectable with subsequently favourable outcome.

HCC is locally invasive and invades vascular structures such as inferior vena cava or portal vein[44]. Autopsy studies indicate that frequent sites of metastasis include lungs, adrenals, bones, diaphragm. CNS as well as direct extension of the tumor through the portal and hepatic venous systems.

Management of hepatoccHu Jar cancer

Several options are available for the treatment of HCC. Because of lack of comparative trials, the choice of treatment is a decision based upon the size and number of the tumors and underlying liver function as well as availability of local expertise and interest.

Surgery

Presently surgical resection offers the only chance of cure for patients with HCC. Unfortunately, only 10% of the patients present with resectable tumors[45]. Upto 90% of the patients have unresectable disease at the time of presentation. Factors that limit resectability include presence of metastases. extensive hepatic involvement specially structures in the porta hepatis and severe cirrhosis. Important factors requiring assessment prior to surgery include measurement of hepatic synthetic function as indicated by semm albumin and bilirubin levels as well as prothrombin time. Cirrhosis alone is not a contraindication to surgery as long as hepatic function is not decompensated. Overall operative mortality ranges between 9-25%. Five year survival rates are 20-30% after resection[46-48]. Recurrence of tumor accounts for nearly 50-90% of the deaths[49].

In general, tumor recurrence is the main cause of poor prognosis in patients operated for HCC. Many factors including tumor size, resection margins and portal vein invasion have been analyzed to prognosticate the risk of tumor recurrence. Patients with tumor size less than or equal to 5 cms or a solitary tumor have a better disease free survival than those with tumor size greater than 5cms or with

multifocal disease. DNA ploidy also affects overall survival. Patients with aneuploid tumors have greater tendency to recur early after hepatic resection as compared to patients with diploid tumors. Those with poor prognostic factors may benefit from multimodality approach and require closer follow-up[50]. Despite improvements in resection techniques such as the ultrasonic dissector and the argon gas coagulator, most patients with HCC remain un resectable. This is usually due to the extent of intra-hepatic disease. It is in these patients that multimodality approach may offer improved chances of survival by reducing intrahepatic disease and increasing the subsequent resectability. In a recent study. 41 hepatic resections were done in 35 consecutive patients from 1985 to 1990. Twenty-one patients had initially resectable tumors. Fourteen patients had initially unresectable lesions. Combination of radiation and chemotherapy was utilized which resulted in a partial response in most of these cases. They subsequently underwent resection. Five year actuarial survival was 45% and 48% for initially resected and those undergoing multimodality therapy. This suggests that some patients with unresectable tumors may become operable with survival rates which are similar to those with initially resectable cancer[51].

Transplantation: This appears to be a rational form of therapy for patients with both decompensating cirrhosis and HCC. The role oforthotopic liver transplantation (OTL) in the treatment of primary hepatic diseases is now widely accepted[52-54]. However, rate of tumor recurrence is high. It is also an extremely expensive approach and necessitates lifelong immunosuppressive therapy. The TNM staging system (Table III). which accounts for tumor size, multiplicity, hepatic lobar involvement, lymph node involvement and extrahepatic disease also correlates with patient survival after OTL[55-58] . Severe hepatic dysfunction, multifocal tumors, bilobar tumors or centrally located tumors are the strongest factors favouring total hepatectonw and OTL over partial hepatectomy. Patients with extra hepatic disease should not be treated by either surgical method and a thorough search for extrahepatic disease must be under-taken before surgical intervention.

Chemotherapy

Treatment of HCC patients with chemotherapy has thus far yielded a low response rate and poor survival. Prognostic factors like performance status, sex, age, presence of jaundice. cirrhosis, etc., are taken into account when predicting survival in clinical trials. Okada et al retrospectively analyzed the significance of different prognostic factors in patients who received systemic chemotherapy inaphase II trial[59]. A performance status of 0-1 (ambulatoiy), tumor size less than 50% of the liver cross sectional area, absence of tumor thrombus in main portal trunk and age less than 60 years were independent favourable prognostic factors. These can be used to classify patients into different prognostic groups with an impact on survival. Design and analysis of future clinical trials should incorporate these prognostic factors.

Single agent chemotherapy: There is little evidence to suggest that any single agent, given systemically, reproducibly has a response rate greater than 25% or has any impact on survival. This includes drugs like 5-Flourouracil, doxombicin, cisplatin, VP-16 and neocarzinostatin[60-62]. In most controlled trials, alkylating agents have been of little use except for intravenous Ifosfomide. In a phase II trial of Ifosfomide in patients with advanced disease (stage lila and IIIb) a response rate of 23% was observed[63]. This drug merits further trials to fully evaluate its potential.

Combination chemotherapy: In general, nothing is gained by adding cytostatics in the management ofHCC. It only leads to increased toxicity. So far none of the combined treatments have given results superior to single agents[64].

Intra-arterial chemotherapy: Most studies of intra-arterial (I/A) chemotherapy require that the patient should have an adequate performance status for placing the catheter, no distant metastasis and adequate liver function. This selects out patients with favourable prognostic factors. Although local tumor shrinkage occurs, considerable toxicity, morbidity, and at time mortality is associated with this

therapy. Hepatic arterial infusion of Flourodeoxiuridine (FUDR), given at doses of 0.3 mg/kg/day for two weeks every month has shown high hepatic tumour regression rate. Itis also associated with severe toxicity limiting the dose to be administered and decreasing the duration of treatment. Intraarterial therapy may also result in progression of the tumor at extra-hepatic sites[65]. Common complications of I/A FUDR include gastritis or duodenal ulcer which develops in upto 50% of patients[66]. It only responds to termination of therapy. Biliaiy sclerosis may also occur and is related to drug-induced cholestatis. It is an irreversible complication[66]. Onset of toxicity may be decreased or delayed by utilizing a lower dose of the drug. Alterations in the I/A FUDR regimen have been proposed to decrease toxicity. These include:

a) Time modified drug delivery involves infusing greatest amount of drug during the periods of maximally expected resistance to toxicity i.e., late afternoon and evening. However, a 33% incidence of biliary toxicity was still observed[67].

b) Pulse infusion of FUDR is still too early to be evaluated.

c) Short cycle FUDR involves escalating doses given for only two days perweek. This work is preliminary and results are yet to be reported.

d) Bolus FUDR has response rates similarto infusionalFUDR but causes less liver enzyme changes.

Biochemical modulation of FTJDR has been done with Leucovonnto augment its efficacy. In a phase I trial of FUDR with Leucovorin. overall response rate was 58%[68].

Pharmacologic modulation of FUDR toxicity has also been tried with co-administration of dexamethasone. A more extensive randomized evaluation of FUDR versus FUDR plus Dexamethasone is in progress. Dipyridamole, an anti-platelet agent, may play a role in limiting the biliary damage from FUDR; a chemical arteritis and microvascular thrombosis resulting in fibrosis. Trials are presently in progress. Other drugs that have been successfully employed as I/A therapy include adriamycin, cisplatin and mitomycin.

External radiation: This has a limited role as doses greater than 3000 rads within three weeks lead to radiation hepatitis.

Novel treatments for unresectable tumors

Therapy for unresectable HCC has generally been unsatisfactory due to poor results with conventional chemotherapy and radiotherapy. However, novel therapeutic approaches appear to offer some promise.

Interruption of hepatic artery blood flow: Liver tumors derive 80% of their blood supply from hepatic artery. Surgical ligation of the hepatic artery leads to preferential ischemia and necrosis of the tumor. However, surgical ligation later leads to the development of collateral vessels thus defeating the aim.

Embolization of hepatic artery can be achieved by using vaso occlusive agents such as starch microspheres, gelfoam, angiostat or polyvinyl alcohol. Repeated occlusions with polyvinyl alcohol has given a response rate of 60%[69]. This therapy, if delivered preoperatively, may allow for simplerand after hepatic resections.

Chemo-embolization is another modality where anti tumour effect of the drug is enhanced by interruption of blood flow resulting in increased local concentration of the drug. It also induces tumor ischemia. Systemic side effects such as nausea and vomiting are also decreased in frequency and severity. A common finding after chemo-embolization is radiological evidence of tumor necrosis. Though not a standard response criteria, this often correlates with pathologic evidence of tumor destruction[70,71].

Enhancement of intra-arterial chemotherapy: This is done by using contrast dye Lipoidol. Lipoidol concentrates in the liver tumor tissue due to its abnormal vascular structure. Tumor necrosis is enhanced and survival improved by emulsifying doxorubicin, mitomycin or cisplatin in lipoidol[72,73] . Five year survival rates of 70% have been reported with Lipoidol treatment followed by surgical

resection[74,75].

Percutaneous intra-tumor alcohol injection: This causes immediate coagulation necrosis due to small vessel damage[76]. It is done under ultrasound guidance. Liver lesions less than 3 cms in diameter have been rendered necrotic. It is specially useful for patients with solitary tumors. Long term utility of this approach and impact on survival remain to be proven in prospective randomized trials.

Cryosurgery: In this technique, cryo-probes are used to circulate liquid nitrogen through the tip which freezes tissue within a 3 ems radius from the trocar. At less than 20 degrees centigrade, most cells (tumor and non-tumor) undergo instant freezing and are killed. It is useful for treating small lesions[77].

Novel radiotherapeutic modalities: Radiolabelled antibodies, like 1131 antiferritin concentrates in HCC due to its increased vascularity. This, alongwith systemic chemotherapy, has been observed to produce a response rate of 48% in patients who are alpba-fetoprotein negative[78,79]. There is, however, significant toxicity due to gamma emmision from iodine isotope. Another radiolabelled isotope yttrium 90 interferes with the Fab end of the antibody and concentrates in hyperplastic liver rather than the primary tumor[80].

Hormonal agents: It has been observed that hepatic tumors express estrogen and androgen receptors[82]. This has prompted the use of agents like tamoxifen (anti-estrogen) and androcur (anti-androgen) as ?otential therapeutic modalities in the treatment of HCC[16,81]. . Results of an EORTC trial are awaited. A recent study suggests an effect of Interferons[83].

Provocative gene therapy: Huber and colleagues reported an innovative approach involving retroviral-mediated, gene-therapy for the treatment of neoplastic diseases[84]. It is called “Virus directed enzyme/prodrug therapy” (VDEPT). This approach exploits the transcriptional differences between normal and neoplastic cells to achieve selective killing of cancer cells. They described this approach for the treatment of HCC.

Replication-defective retroviruses were constructed containing a varicella-zoster virus thymidine kinase (VZV TK) gene that is transcriptionally regulated by either the hepatoma-associated aipha-fetoprotein or liver associated albumin transcriptional regulatory sequences. After retroviral infection, expression of VZV TK was linked to either aipha-fetoprotein or albumin-positive cells. VZVTK metabolically activates the non-toxic prodrug 6- Methoxypurine arabinonucleoside (ara M) which ultimately leads to the production of toxic metabolite adenosine arabinonucleoside tnphosphate(ara ATP). Cells that selectively express VZVTK become selectively sensitive to am M due to the VZV TK dependent metabolism of ara M to am ATP. Hence, these retroviral derived genes generated tissue specific expression of VZV TK, tissue specific metabolism of am M to am ATP and tissue specific cytotoxic effect on cultured HCC cells.

Conclusions

HCC is a preventable disease. Vaccination against HBV decreases the incidence of HCC. Additionally, poor prognosis associated with HCC may be improved ifitcanbe detected and treated at an early stage. This can be achieved by utilizing AFP and ultrasonography in high risk patients. Forthose with more advanced disease, several modes of treatment are available with the potential to improve survival and ability to provide significant palliation. Multimodality therapies are generally more effective. Proper selection of patients and therapy require understanding of biology of the tumor, intm-tumoml blood flow, tumor extent and hepatic function[85]. Novel therapeutic approaches for patients with unresectable HCC have promising initial results. Further trials are indicated.

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