EVALUATION OF CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA"** is a bonafide work done by **DR. B. MAHADEVAN**, during the study period 2006-2009 and is being submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the requirements for the award of DM Branch IV Medical Gastroenterology Degree.

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DECLARATION

I declare that this dissertation entitled **"EVALUATION OF CLINICAL AND LABORATORY PARAMETERS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA"** has been done by me under the guidance and supervision of **Prof. Mohammed Ali, MD, DM.** It is submitted in partial fulfillment of the requirements for the award of DM Gastroenterology degree by The Tamilnadu Dr. M.G.R. Medical University, Chennai. This has not been submitted by me for the award of any degree or diploma from any other University.

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CONTENTS

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem responsible for fifth most common neoplasm in the world, and third most common cause of cancer related deaths.[i] Age adjusted incidence is 5.5 – 14.9 per 100,000 population worldwide. Age adjusted incidence for HCC in developing countries are two to three fold higher than those in the developed countries.[ii] Almost 80% of liver cancers occur in developing countries like Asia and Africa.[iii] A rise in the incidence of mortality from HCC has been observed in different countries.[iv] Approximately 77% of deaths from HCC occur in developing countries.

The prognosis of HCC is dismal with 5-year survival being 1–4%.[3] Global distribution of HCC is very variable. Most Western countries have a low HCC incidence (<5 cases/ y/100,000), but most Asian countries have an intermediate (5–15 cases/y/1,000,000) or high (>15 cases/y/100,000) incidence of HCC. [v] Low incidence of <5 cases/y/100,000 of population has been reported from India.[vi] This low incidence is in contrast with the widespread contamination of foods with mycotoxins and the moderately high prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) related chronic liver disease in India, which are considered as the most important risk factors for the development of HCC worldwide.

In India, the mean incidence of HCC in four population-based registries is 2.77% for males and 1.38% for females. The prevalence of HCC in India varies from 0.2% to 1.6%.[vii] Hepatitis B virus infection is the most common etiologic factor in high incidence areas, while hepatitis C infection is more prevalent in the low incidence areas. [viii] [ix] Unlike other low incidence zone, in India HBV is the main etiological factor associated with HCC. [x] [xi] [xii] In the west, majority of HCC are diagnosed incidentally during routine evaluation. However, in India, most of the patients in clinical practice present at an advanced stage ruling out curative treatment in most cases.

Despite India being a low incidence zone for HCC, the estimated HCC cases in 2001 was 12 750.[7] However, there is paucity of published literature on profile of HCC patients in India, making formulation of a proper health care strategy difficult. Most of the published literatures were retrospective studies and moreover limited number of studies available for South Indian population. Hence we have undertaken this study to analyze the characteristics of HCC, especially with regard to their clinical, etiological, radiological and cytohistological profile.

AIMS & OBJECTIVE OF THE STUDY

The study was conducted with the objective of

a) To study the clinical, etiological, radiological and cytohistological profile in patients with Hepatocellular carcinoma
b) To identify the association between serum alpha fetoprotein with stage of the disease.

REVIEW OF LITERATURE

Hepatocellular carcinoma (HCC) represents more than 5% of all cancers in the world, and the estimated number of cancer-related deaths exceeds 500,000 per year. [xiii] Most patients who have HCC are diagnosed at advanced stages leading to an overall 1-year survival of 25%.[xiv] With the significant increase in the number of patients who have HCC, early detection and treatment of this tumor are vital to improve outcomes.[xv]

Epidemiology

Worldwide Distribution

Liver cancer burden is not distributed evenly throughout the world. According to the age adjusted HCC incidence per 100 000 population per annum, different geographic regions can be divided into three incidence zones: low (<5), intermediate (between 5 and 15), high (>15).[5] More than 80% of HCC cases occur in either sub-Saharan Africa or in Eastern Asia. China alone accounts for more than 50% of the world's cases (men, 35.2/100,000; women, 13.3/100,000). North and South America, and Northern Europe found to have low incidence (<5.0/100,000) of liver cancer among most populations. United Kingdom (male, 2.2/100,000; female, 1.1/100,000), and Australia (male, 3.6/100,000; female, 1.0/100,000) also noted low tumor burden. [xvi] Neonatal vaccinations against hepatitis B virus (HBV) and shift the staple diet from corn to rice (limit the exposure to aflatoxin B1 lowered the incidence of HCC in Asian countries.[xvii xviii]

Race/Ethnicity

HCC incidence rates vary greatly among different populations living in the same region. In United States, HCC rates are 2 times higher in Asians than in African Americans, whose rates are 2 times higher than those in whites. The reason for this ethnic variability

likely includes differences in the prevalence and acquisition time of major risk factors for liver disease and HCC.[16]

Sex

In almost all populations, males have higher liver cancer rates than females, with male: female ratios usually averaging between 2:1 and 4:1. At present, the largest discrepancies in rates (>4:1) are found in medium-risk European populations.[xix] The reasons for higher rates of liver cancer in males may relate to sex-specific differences in exposure to risk factors. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores. Several studies conducted in Taiwan[xx] [xxi] reported a positive association between increased circulating testosterone levels and HCC in HBV-infected men.

Age

The global age distribution of HCC varies by region, incidence rate, sex, and by etiology.[16] In all areas, female rates peak in the age group 5 years older than the peak age group for males. In low-risk populations (United States, Canada, and United Kingdom), commonly occurs among persons aged 75 and older. A similar pattern is seen among most high-risk Asian populations (Hong Kong and Shanghai). In contrast, male rates in high-risk African populations tend to peak between ages 60 and 65, whereas female rates peak between 65 and 70. These variable age specific patterns likely are related to differences in the dominant hepatitis virus in the population, the age at viral infection, and the existence of other risk factors.[19]Although most HCV infections acquired in adulthood, but most HBV carriers became infected at very young ages.

Distribution of Risk Factors

In low-rate HCC areas, the increasing number of persons living with cirrhosis is the likely explanation for the increasing incidence of HCC. Combination of factors including an

increasing incidence of cirrhosis caused by HCV, HBV infection, as well as an improvement in survival among cirrhosis patients.[19]

In most high-risk areas, the dominant risk factor is chronic HBV infection. In Asia, HBV infection largely is acquired by maternal-child transmission, whereas sibling-to-sibling transmission at young ages is more common in Africa. Consumption of AFB1 -contaminated food is the other major HCC risk factor in most high-rate areas.

By contrast, in Japan, Egypt and in Southern Europe, HCV is the main cause of HCC which occurs in older patients, nearly all of them with advanced fibrosis or cirrhosis. In Northern and Central European countries, HCV infection and alcohol are the main causes of cirrhosis. In France, ethanol is still the leading cause of cirrhosis and was responsible for 60% of all HCC cases.[xxii]

HCC in Asia

In China and Taiwan, almost one-fifth of the populations are carriers of HBV, and the majority of persons with HCC are HBsAg-positive. [xxiii] Although HCV infection is the main risk factor in HbsAg negative HCC, HCV prevalence is low, with only 0.9% of healthy blood donors positive for anti-HCV.[xxiv] In contrast to the rest of Asia, cases of HCC in Japan are mainly related to HCV infection, and its incidence is rising but on a larger scale.[xxv] The reasons underlying this difference are likely related to the wide transmission of HCV to young people in Japan from contaminated blood and needles after the Second World War. It also seems that in Japanese patients with chronic viral hepatitis, the progression to HCC occurs at an accelerated rate in HCV infection compared with HBV infection.

HCC in India

Most Asian countries are in intermediate or high incidence zones of HCC. In India, the mean incidence of HCC in four population-based registries is 2.77% for males and 1.38% for females. HCC accounted for 1.9% of the 24,975 cases of cancers recorded at 6 registries put together; the proportion ranging from 1.1% (94/8763) in Delhi to 5.3% (10/187) in Barshi rural registry. [xxvi], [xxvii] The prevalence of HCC in India varies from 0.2% to 1.6%.[7, 27] Unlike other low incidence zone, in India HBV is the main etiological factor associated with HCC.[10, 11, 12] However, in India, most of the patients in clinical practice present at an advanced stage ruling out curative treatment in most cases. A prospective study by Paul et al[xxviii] revealed the estimated incidence of HCC among cirrhotic patients was 1.6% per year.

Risk Factors and Pathogenesis of HCC

Hepatocellular carcinoma is multifactorial in etiology and complex in pathogenesis. Several risk factors have been described in various studies.

Risk Factors for Hepatocellular Carcinoma

Major Risk Factors

- Chronic hepatitis B virus infection
- Chronic hepatitis C virus infection
- Cirrhosis
- Dietary exposure to aflatoxin B1

Minor Risk Factors

- Oral contraceptive steroids
- Cigarette smoking
- Dietary iron overload in persons of black African ancestry
- Hereditary hemochromatosis
- Wilson disease
- α1-Antitrypsin deficiency
- Type 1 hereditary tyrosinemia

- Type 1 and type 2 glycogen storage disease
- Membranous obstruction of the inferior vena cava

Hepatitis B Virus (HBV)

Globally, HBV is the most common cause of HCC, with an estimated 300 million persons with chronic infection worldwide. Case-control studies have shown that chronic HBV carriers have a 5- to 15-fold increased risk of HCC compared with the general population. The great majority, between 70% and 90%, of HBV-related HCCs develop in patients with cirrhosis. However, HCC may occur in HBV infected individual in the absence of cirrhosis.

The risk of HCC in patients with chronic HBV infection depends on age, carrier status, inflammation, presence of cirrhosis and the family history of HCC.[xxix] Prospective studies have shown that the annual incidence rate is between 2.2% and 4.3% for patients with HBV cirrhosis, between 0.1% and 1% in patients with chronic hepatitis, and between 0.02% and 0.2% in inactive carriers.[xxx]

The increased HBV related HCC risk associated where HBV is endemic. In these areas, vertical transmission is the usual mode of acquiring infection seen up to 90% of chronic infected persons. This pattern is different in areas with low HCC incidence rates where HBV is acquired in adulthood through sexual and parenteral routes (horizontal transmission); with only 10% become chronic HBV carriers. The annual HCC incidence in chronic HBV carriers in Asia ranges between 0 .4% and 0.6%.

The HBsAg positivity in Indian HCC patients varies from 36% to 74%. [10, 11, xxxi] India despite being in an intermediate endemic zone for HBV has low incidence of HCC unlike other Asian countries. The relative risk of developing HCC in Indian patients with chronic HbsAg infection was estimated to be 7.8 from various studies.[xxxii] The prevalence of Hepatitis B and C infection in HCC patients is as follows in table 1.

Groups at high risk for HCC among Hepatitis B carriers are Asian males > 40 years

old, Asian females > 50 years old, cirrhosis, family history of HCC and patients who do not have cirrhosis: depends on viral genotype, viral replication

Table 1 Prevalence of hepatitis B and C in HCC

Place	No of patients	HbsAg + ve (%)	Anti HCV + ve (%)
Sarin et al 2001, Delhi[12]	74	71	4
Joshi et al 2003, Hyderabad[xxxiii]	40	47.5	20
Saini et al 2006, Chandigarh[xxxiv]	47	54	27
Kumar et al 2008, Delhi[xxxv]	246	73	15

and inflammatory activity.[29] HCC risk also is increased in patients with higher levels of HBV replication (HBeAg positivity and high HBV-DNA levels).

Spontaneous or treatment-induced development of antibodies against hepatitis B surface antigen and HBeAg, found to have improved clinical outcomes against HCC. A meta-analysis of 12 studies, patients with chronic HBV infections treated with or without interferon therapy followed up for 5 years found a lower HCC incidence in treated than untreated patients.[xxxvi] However, this difference was not statistically significant. Occult HBV infection has been associated with increased risk of HCC, but this fact is not proven yet. The risk of HCC is substantially lower in persons who are immune to HBV. Beasley found that the incidence of HCC was significantly lower in immune persons compared with carriers (5 vs 495 per 100,000 per year). [xxxvii]

Hepatitis B vaccination is widely recognized as the most effective measure to prevent HBV infection and HBV-associated complications, including HCC. The first evidence came from Taiwanese study[xxxviii] showed that HCC is preventable via effective vaccination. The average incidence of HCC declined from 0.7 (1982 to 1986) to 0.36 (1990 to 1994) per 100,000 children. And there was a similar decline in the mortality associated with HCC.

Integration of HBV DNA into host's genome may activate cellular proto-oncogenes or

suppress growth-regulating genes in cis. HBV-related tumors have a distinct pattern of genetic mutation with greater chromosome instability than HCC with higher prevalence of loss of heterozygosity has been correlated with tumor aggressiveness. The HBV X gene encodes a viral protein that plays a central role in HBV infection and in hepatocarcinogenesis. The HBx protein is a transcriptional factor that can alter the expression of many cellular genes, including oncogenes such as c-myc and c-myb, as well as tumor suppressor genes such as APC, p53, p2.[xxxix] The X protein has the ability to block p53 mediated apoptosis in vivo, which contributes to the development of preneoplastic and neoplastic hepatocytes. It has been also demonstrated that core promoter mutations, T1653 mutations, HBV DNA levels ≥4log10 copies/mL and presence of cirrhosis were independent factors for the development of HCC.[xl]

HBV-related HCC is predominant in male, with a male to female ratio of 5–7:1. This is attributed to the elevated androgen level and the enhanced androgen receptor (AR)-mediated activity in the host. HBx is a noncellular positive coregulator for androgen receptors makes males vulnerable to HBV infection and the subsequent development of cancer.[xli] HBx protein may play a significant role in inducing the expression of angiopoietin-2; contribute to pathological angiogenesis and hepatocellular carcinoma progression.[xlii]

Chronic Hepatitis C

Chronic HCV infection is the emerging cause of HCC in industrialized countries with the reported prevalence of more than 80% in Japan, Italy and Spain. There is geographic variability in the prevalence of markers of HCV infection in patients who have HCC; it has ranged from 27% in the United States, from 27%s to 75% in Western Europe and up to 80% to 90% in Japan.[xliii] The risk of HCC in patients with chronic hepatitis C is present mainly in patients with established cirrhosis, in whom the incidence of HCC is between 2 and 8% per

year.[xliv] A large community-based prospective study from Taiwan that included 12,008 patients observed a 20-fold increased risk for developing HCC in anti–HCV-positive patients when compared with anti–HCV-negative subjects.[xlv] The estimated risk of developing HCC depicted in Figure 1 based on study by Hassan et al. [xlvi] In Chronic HCV-infected patients, host and environment factors appear to play more important role than viral factors in determining progression to cirrhosis. These factors include older age, older age at the time of acquisition of infection, male sex, heavy alcohol intake (>50 g/day), diabetes, obesity, and co-infection with human immunodeficiency virus or HBV.[14] HCV viral factors such as genotype, load, or quasispecies are not important in determining the risk of progression to cirrhosis or HCC. Successful antiviral therapy in patients with HCV related cirrhosis may decrease HCC risk moderately among patients treated with interferon.[xlvii] [xlviii]

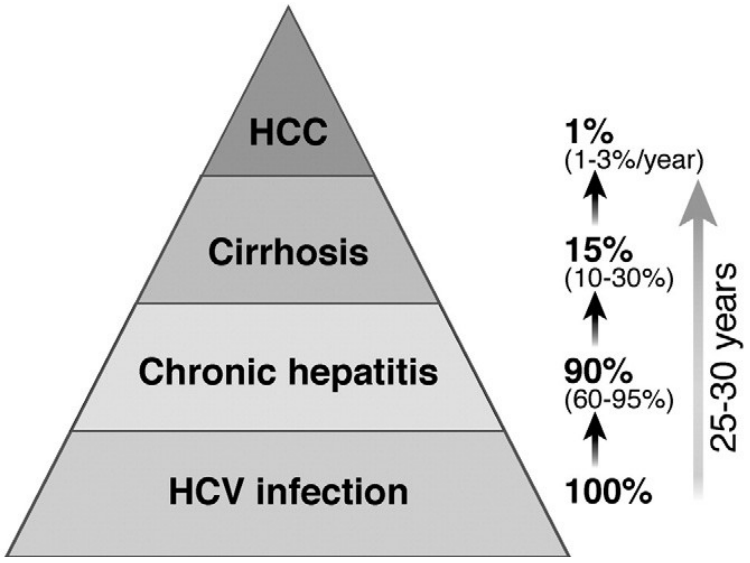


Figure 1 Proportion of patients with HCC related to HCV viral infection.

Unlike HBV, HCV is an RNA virus that does not integrate into the host genome and host-viral protein. At least four of the HCV gene products HCV core, NS3, NS4B and NS5A, alter several potentially oncogenic pathways. It has been demonstrated that HCV Core could contribute to viral persistence by regulating anti-apoptosis factors that could prevents apoptosis and enhance the survival of HCV infected host cells. The HCV core protein regulates transcription of different cellular genes, including the proto-oncogene c-myc, suggesting its involvement in the deregulation of normal cell

growth. HCV core protein would be involved in hepatocarcinogenesis through two different molecular pathways; 1) the core protein would act on the function of mitochondria, leading to oxidative stress, which yields genetic aberrations in cell growth-related genes[xlix] 2) Modulation of gene expressions and intracellular signal transductions. The combination of these alterations would provoke the development of HCC in HCV infection.[49]

HCV nonstructural proteins NS3 and NS5A were shown to possess direct oncogenic potential. Positive correlation identified between COX-2 and iNOS expression with hepatic angiogenesis in HCV-positive HCCs, suggesting role in tumor angiogenesis. Two phenotypes such as angiogenic and MDR (Multi-Drug Resistance) have been described. Both phenotypes are correlated to aggressiveness of cancer and prognosis of patients.[l] MDR phenotype in cancer cells express a glycoprotein (P-gp) that binds hydrophobic molecules, including chemotherapic agents, and exports them out of the cell by ATP hydrolysis.[li] This favors tumor cell survival and proliferation. Induction of MDR phenotype is associated with an up-regulation of the COX-2 and iNOS in human HCC cell lines. COX-2 is associated with different cellular functions and is over expressed in several human tumors. PGs and NO play an important role in tumor growth, angiogenesis. Evidence suggested that the MDR and the angiogenic phenotypes are linked to each other in human liver cancer cells.[lii]

Co-infection with HIV:

Patients co-infected with HIV and either hepatitis B or hepatitis C may have more rapidly progressive liver disease and when they reach cirrhosis they are also at increased risk of HCC. The MORTAVIC study indicated that HCC was responsible for 25% of all liver deaths in the post-HAART era.[liii]

Treated chronic HBV and HCV infections

There is no convincing evidence that interferon treatment of chronic hepatitis B

reduces the incidence of HCC. Studies which shown in decrease reduction in the incidence of HCC with lamivudine or interferon therapy, the event rate was low. A meta-analysis conducted in patients with chronic HCV infection concluded that the benefit with interferon treatment was mainly seen in those who achieved sustained virological response, however the effect was small. The steps required to initiate the carcinogenic pathway probably occur many years before the disease becomes inactive, and so the threat of HCC remains even if fibrosis decreases. Regressed fibrosis is not a rationale to withhold surveillance.[29]

Cirrhosis

Most often HCC occurs within an established background of chronic liver disease and cirrhosis (70%–90% of all detected HCC cases). Major causes of cirrhosis in patients with HCC include hepatitis B, hepatitis C, alcoholic liver disease, and possibly nonalcoholic steatohepatitis. Less common causes include hereditary hemochromatosis, alpha-1 antitrypsin deficiency, autoimmune hepatitis, and some porphyrias.

Cirrhosis is macronodular and is often attributed to chronic HBV infection in Chinese and African populations, whereas in other populations, cirrhosis is commonly mixed macronodular and micronodular. Micronodular cirrhosis may results from chronic HCV infection, alcohol abuse, or both. Cirrhosis contributes to hepatocarcinogenesis mainly by acting as a potent tumor promoter. Incidence of HCC in compensated cirrhosis enumerated in table 2. Male sex, age, and duration of cirrhosis are the major risk factors for hepatocellular carcinoma in cirrhotic patients.

Table 2 Incidence of HCC in Patients with Compensated Cirrhosis

	Fattovich et al,[liv] Italy	Hu and Tong,[lv] United States	Serfaty et al.[lvi] France	Paul et al[28] India
Number of patients	384	112	103	301
Follow-up period, y	5.0	4.5	3.3	4.0
HCC, % per year	1.4	2.3	3.3	1.6

Aflatoxin

AFB1 is a mycotoxin produced by the *Aspergillus* fungus. This fungus grows readily on foodstuffs such as rice, corn and peanuts stored in warm, damp conditions. AFB1 is a powerful hepatocarcinogen, leading the International Agency for Research on Cancer to classify it as carcinogen. After ingestion AFB1 is metabolized to an active intermediate, AFB1 -*exo*-8, 9-epoxide, which can bind to DNA and cause mutation in the p53 tumor-suppressor gene. This mutation observed in 30%–60% of HCC tumors in aflatoxin-endemic areas.[lvii] Strong evidence of AFB1 is a risk factor for HCC based on epidemiologic studies.

Short-term prospective studies have analyzed the interaction between AFB1 exposure and chronic HBV infection. It has been found that aflatoxin and HBV infection exposure increases 4-fold and 7-fold risk of HCC respectively. However, individuals exposed to both showed 60-fold increased risk of HCC.[lviii] In most areas where AFB1 exposure is a problem, chronic HBV infection also is highly prevalent.

A study [lix] from Indian subcontinent reported the prevalence of aflatoxin B1 (AFB1) in 31 liver biopsies and 7 liver-resection specimens from histopathologically proven HCC. 58.1% of HCC cases showed AFB1 in liver biopsies. Positive for AFB1 in liver biopsy noted in 46.1% of HBsAg-positive patients, which proves that aflatoxin have a significant association with HCC.

Minor risk factors

Oral Contraceptives: Studies showed a significant 2- to 20-fold increase in HCC risk with longer durations (>5 y) of oral contraceptive (OC) use. This has been reported from countries with low incidence of HCC. Nuclear estrogen receptors exist in hepatocytes and estrogens are thought to cause liver neoplasia by increasing proliferation rates, thereby increasing rates of spontaneous mutations. OC use also has been linked to malignant liver tumors such as mixed hepatocellular and ductal carcinoma, cholangiocarcinoma, and hepatoblastoma.[50]

Hemochromatosis: A population-based study conducted in Sweden using multiple national data sources indicated a 1.7-fold increase in the incidence of HCC among 1800 individuals with hereditary hemochromatosis. Malignant transformation common observed in the presence of cirrhosis, but this has been reported in patients without cirrhosis. It is possible that excessive free iron in tissue may be carcinogenic by generating mutagenic reactive oxygen species. [lx]

Conflicting evidence observed between cigarette smoking and the occurrence of hepatocellular carcinoma based on epidemiologic studies. But most of the evidence suggests that smoking is a minor risk factor. Heavy smokers have a 50% higher risk than that of nonsmokers. The cytochrome P450 enzyme system responsible for the metabolic activation of a number of chemical carcinogens is highly inducible by smoking.[lxi]

Hepatocellular carcinoma develops in nearly 40% of patients with membranous obstruction of the inferior vena cava, reported in Asian and African countries. Repeated hepatocyte necrosis followed by regeneration resulting from the severe and unremitting hepatic venous congestion makes the cells susceptible to environmental mutagens, as well as to spontaneous mutations.[lxii]

Clinical features

In advanced disease, patients with hepatocellular carcinoma often present with typical symptoms and signs. Clinical recognition often is difficult in early stages. The clinical picture is very variable

Asymptomatic Presentation

Since the easy availability of imaging techniques, increasing numbers of cirrhotic patients are being diagnosed with HCC at asymptomatic stage. These tumors tend to be smaller with current imaging methods, tumors as small as 0.5 cm can be detected with newer techniques. Therefore these tumors are more amenable to potentially curative therapies such as resection, transplantation, and tumor ablation. The frequency of asymptomatic diagnosis is dependent on the intensity of the screening on high risk patients. In a series of 461 Italian patients, asymptomatic HCC was detected in 23%. [lxiii]

Hepatic Decompensation

Another common scenario for the presentation of HCC is sudden hepatic decompensation in a patient known to have cirrhosis. New-onset ascites, recurrent variceal hemorrhage, or progressive encephalopathy should always raise suspicion for HCC. The ascites may be difficult to control with standard diuretic therapy and often is bloodstained.

Gastrointestinal Hemorrhage

Approximately 10% of patients have gastrointestinal bleeding at presentation. In 40% of these patients, the bleeding is the result of esophageal varices resulting from portal vein invasion and elevated portal pressure. Peptic ulcer disease and other benign causes account for the remaining 60% of cases involving bleeding. Rarely, the tumor directly may invade the

gastrointestinal tract and causes bleeding.

The “Classic Triad”

In clinical practice, HCC often present with the triad of right upper quadrant abdominal pain, weight loss, and hepatomegaly. Patients with these symptoms at presentation usually have a tumor larger than 6 cm. The pain frequently is described as a dull continuous ache that intensifies late in the course of the illness due to involvement of Glisson’s capsule. The pain may be referred to the shoulder. Firm, often massive, nodular hepatomegaly is an invariable feature of symptomatic HCC. An arterial vascular bruit due to increased vascularity may be a useful diagnostic pointer. It is observed in 25% of cases, occurs in systole, rough in character, and is not affected by changing the position. Although not pathognomonic, it rarely occurs with hepatic metastases.[lxiv]

Tumor Rupture: “Hemoperitoneum”

Spontaneous rupture is a rare and catastrophic complication of HCC that may occur if a large vascular tumor on the periphery of the liver. It may occur spontaneously or with minor blunt abdominal trauma. The clinical presentation is that of severe abdominal pain, vascular collapse, and signs of peritoneal irritation. Although hemoperitoneum is a frequent event late in the course of the disease, it is a presenting feature in less than 5% of cases. The diagnosis is established by paracentesis, which reveals bloodstained fluid. Angiography and embolization of the bleeding vessel can be an effective method for managing this life-threatening complication.

Extrahepatic Endocrine and Paraneoplastic Syndromes

These systemic sequelae result from synthesis and secretion of biologically active substances by the tumor. Advances have been made in understanding the mechanisms underlying some of these paraneoplastic phenomena. Less than 5% of patients results

hypoglycemia. Type A hypoglycemia is a milder form of glycopenia that occurs in the terminal stages of hepatocellular carcinoma due to increased demands for glucose by a large rapidly growing tumor. Type B hypoglycemia is believed to result from the defective processing by malignant hepatocytes of the precursor to insulin-like growth factor II (pro-IGF-II). Polycythemia (<10% of patient) is caused by synthesis of erythropoietin by the tumor.

Patients with sclerosing type of HCC may present with hypercalcemia in the absence of osteolytic metastases. The probable cause is secretion of parathyroid hormone-related protein by the tumor. Arterial hypertension complicating HCC is the consequence of ectopic synthesis of angiotensinogen by malignant hepatocytes. Feminization results from the tumor's conversion of circulating dehydroepiandrosterone to estrone. Hypercholesterolemia is the result of de novo synthesis of cholesterol by the tumor. Watery diarrhea is occasionally severe and intractable, probably is related to secretion of peptides that promote intestinal secretion such as vasoactive intestinal peptide, gastrin, and prostaglandins. Cutaneous manifestations are not specific for the diagnosis of HCC. It includes dermatomyositis, pemphigus foliaceus, sign of Leser-Trelat, pityriasis rotunda, and porphyria cutanea tarda.

Paraneoplastic Syndromes Associated With HCC[lxv]

- Hypoglycemia
- Polycythemia (erythrocytosis)
- Hypercalcemia
- Sexual changes: isosexual precocity, gynecomastia, feminization
- Systemic arterial hypertension
- Watery diarrhea syndrome
- Carcinoid syndrome
- Osteoporosis
- Hypertrophic osteoarthropathy
- Thyrotoxicosis
- Hypercholesterolemia
- Thrombophlebitis migrans
- Polymyositis
- Neuropathy
- Cutaneous manifestations: pityriasis rotunda, Leser-Trelat sign, dermatomyositis,
- Pemphigus foliaceus, porphyria cutanea tarda

Other Rare Manifestations

Fever of unknown origin may be a manifestation of HCC. Massive tense ascites resulting from hepatic vein spread (Budd–Chiari syndrome) and obstructive jaundice resulting from bile duct compression are two complications of locally advanced tumor. Other rare presentations include bone pain (skeletal), sudden paraplegia (vertebral destruction), and cough or dyspnea (multiple pulmonary metastases).

Diagnostic Methods

A diagnostic approach to HCC has been developed based on the literature and expert consensus and incorporates serology, cytohistology, and radiologic characteristics. Diagnosis of HCC can be confidently established by (1) a focal hepatic mass >2 cm is identified on one imaging technique wherein characteristic contrast enhancement features on the arterial phase with venous washout on an MRI or CT can be demonstrated; (2) a focal hepatic mass with atypical imaging findings (no arterial enhancement with washout), or a focal hepatic mass detected in a noncirrhotic liver, should undergo a biopsy. [lxvi] Diagnostic criteria formulated based on conclusion of the Barcelona-2000 EASL conference[lxvii]

On the other hand, the recommended diagnostic approach for tumors >2 cm or tumors that do not meet above criteria is such that (1), when nodules within 1–2 cm on screening of a cirrhotic liver are typical of HCC (hypervascular with washout) on 2 imaging modalities, the lesion should be treated as HCC. In an atypical lesion where the vascular profile is not consistent among techniques, a biopsy of the lesion should be considered. (2) Nodules smaller than 1 cm should be followed with US at 3- to 6-month intervals.

Serum Markers

α-Fetoprotein

A large number of candidate markers have been advocated during the last 40 years, but none is more helpful than the first one described α-fetoprotein (AFP). AFP is a glycoprotein that normally is produced during gestation by the fetal liver and yolk sac. Normally, it is present in high concentration in the fetal serum. AFP is elevated in approx 60–70% of patients with HCC. [lxviii] The normal range of this serum marker is 0–10 ng/mL, and levels higher than 400 ng/mL are diagnostic of HCC.[67] False-positive results may be caused by acute and chronic benign hepatic diseases with a high necroinflammatory activity, germ cell tumors, or pregnancy. The sensitivity, specificity, and positive predictive value of AFP in three well-performed screening studies for HCC ranged from 39 to 64%, 76 to 91%, and 9 to 32%, respectively.[lxix] AFP production is age-related. Younger patients are more likely to have raised levels and to attain very high concentrations. There is no obvious correlation between serum AFP concentrations and any clinical or biochemical indices or the survival time after diagnosis. Because of both false-positive and false-negative results, serum AFP falls short of being an ideal tumor marker. Thus, a number of alternative substances have been suggested, although none have proved to be more useful than AFP.

Several attempts have been made to improve the HCC specificity of AFP by measuring particular glycoforms of the protein. These isoforms have differential affinities for lectins such as Lens culinaris agglutinin and concanavalin A. Lens culinaris-reactive AFP, also known as AFP-L3, may be superior to total AFP as a marker of HCC. Other glycoforms, such as mono- and disialylated AFP, may also have increased sensitivity in HCC detection. However, data supporting their superiority over conventional AFP measurements are lacking.

Fucosylated AFP

AFP is heterogeneous in structure with differences in its asparagine-linked oligosaccharide side chain. The resultant differential reactivity with lectins is used in

diagnosis. Reactivity with lens culinaris agglutinin A is helpful in distinguishing HCC from benign hepatic diseases and also to differentiate between HCC and other AFP-producing tumors. The test results are positive in approximately 35% of patients with HCC tumors smaller than 2 cm, and this isoform of AFP may be present in serum up to 9 months before the detection of HCC by other methods.

Des-γ-carboxy Prothrombin

Malignant hepatocytes seem to lack the ability to carboxylate glutamic acid to form γ-carboxyglutamic acid. The resulting abnormal prothrombin has been referred to as des-γ-carboxyprothrombin (DCP). Because this is the same prothrombin formed by vitamin K absence or antagonism, DCP is also known as PIVKA-II. Although DCP has demonstrated a greater specificity than AFP, it still lacks sensitivity, especially for HCC tumors less than 3 cm in diameter, with sensitivity ranging from 19 to 48%. Compared with AFP, DCP levels had higher sensitivity and specificity in differentiating HCC from nonmalignant chronic liver disease. One prospective study screening cirrhotic patients for HCC, using cutoff values of 40 ng/mL for AFP and 80 mAU/mL for DCP, showed 65% sensitivity and 85% specificity when both markers were combined.[lxx]

α-L-Fucosidase

Alpha-L-fucosidase is a expressed lysosomal enzyme whose activity is detectable in the sera of healthy subjects. It might be useful as a complementary assay in conjunction with AFP. The disease specificity of the a-L-fucosidase assay is limited since non-cancerous, extrahepatic diseases such as diabetes, pancreatitis, and hypothyroidism are associated with elevated serum activities. No quantitative serum assays are currently available, and the diagnostic potential of this marker remains unclear. Sensitivity and specificity of various tumor markers are given in table 3.

Table 3 Sensitivity and specificity of various tumor markers

Marker	Sensitivity (%)	Specificity (%)
Alpha-fetoprotein	High-incidence populations: 80-90 Low-incidence populations: 50-70	90
Des-γ-carboxyprothrombin	58-91	84
α-L-Fucosidase	75	70-90

Glypican-3

Glypican-3 (GPC-3) is a cell-surface glycoprotein that is absent in hepatocytes of healthy subjects and patients with hepatitis, but highly expressed in hepatocellular cancer cells. GPC-3 is normally involved in the regulation of cell proliferation and survival during embryonal development and functions as a tumor suppressor. It is detectable in the serum in at least 50% of patients with HCC. Although still experimental, it is of interest because it seems to be expressed preferentially in small HCC tumors compared with larger HCC tumors.

Other Markers

Other markers of HCC that have been studied include tumor-associated isoenzymes of γ-glutamyl transpeptidase, urinary TGF-β-1, serum levels of circulating intercellular adhesion molecule (ICAM) -1, Insulin-like growth factor-II (IGF-II), Insulin-like growth factor-binding protein-2 (IGFBP-2), Human cervical cancer oncogene (HCCR), Golgi protein 73, Hepatocytes growth factor (HGF), KL-6, serum proteomics and HCC-specific auto-antibodies. None of these diagnostic tests have demonstrated superior accuracy compared with serum AFP. Two tumor markers, abnormal vitamin B12-binding protein and neurotensin have been

linked specifically to the fibrolamellar variant of HCC.

Diagnostic Imaging

Once a screening test is abnormal or there is a clinical suspicion that a patient may have HCC, imaging is very important for the diagnosis and staging of this tumor.

Ultrasonography

Ultrasonography often is used as a screening method for high-risk patients and is repeated at frequent intervals. A small HCC may be hypoechoic, hyperechoic, or isoechoic on sonography. The ultrasonographic appearance is influenced by the presence of fat, calcium, and necrosis. Advantages of ultrasonography include safety, availability, and cost effectiveness, though it is operator dependent. Approximately two thirds of symptomatic hepatocellular carcinomas are uniformly hyperechoic, whereas the remainder is partly hyperechoic and partly hypoechoic.[lxxi] Ultrasonography with Doppler technology is useful for assessing the patency of the inferior vena cava, portal vein and its larger branches, hepatic veins, and biliary tree.[71]

CT scan and MRI

The most reliable diagnostic tests are triple-phase helical CT and triple-phase dynamic contrast enhanced magnetic resonance imaging (MRI)[lxxii] whereas hepatic angiography has fallen out of favor in most practice settings. HCC derives its blood supply predominantly from the hepatic artery, whereas the remainder of the nontumorous liver receives both arterial and portal blood. The hallmark of HCC during CT scan or MRI is the presence of arterial enhancement followed by delayed hypointensity of the tumor in the portal venous and delayed phases (washout).[lxxiii] The presence of arterial enhancement followed by washout has a sensitivity and specificity of 90% and 95%, respectively. However, 71% of patients with

HCC will have arterial enhancement and whereas the rest do not have these features and, therefore, will require liver biopsy for the diagnosis of HCC. Studies have compared the accuracy of CT and MRI for HCC diagnosis by using the explanted liver as the gold standard[lxxiv]. (Table 4) These show that MRI is slightly better in the characterization and diagnosis of HCC when compared with CT scan. The performance of CT and MRI is affected by the size of the lesions. Tumors larger than 2 cm, MRI are reported to have accuracy >90%; however, in tumors smaller than 2 cm, this level is reduced to 33%.[lxxv]

Table 4: Comparison in the Accuracy of CT scan and MRI Scan in HCC

Author	Gold standard	No. patients	No. nodules	HCC (n)	CT scan (Sens/ Spe)	MRI (Sens/ Spe)
Burrel et al[74]	Explanted liver	50	127	76	61/66	76/75
Libbrecht et al[75]	Explanted liver	49	136	77	50/79	70/82

Hepatic Angiography

With the advent of CT and MRI, the diagnostic role of hepatic angiography has decreased. Current role of angiography is to delineate the hepatic arterial anatomy in planning surgical resection, liver transplantation, embolization or chemoembolization of the tumor, or infusion of cytotoxic drugs. The arteries in the tumor are irregular in caliber and the smaller branches may show a bizarre pattern. The hepatic veins fill early, and retrograde filling of the portal veins results from the presence of arteriovenous anastomoses within the tumor.

Pathology

Macroscopic Pathology

Most HCCs arise in cirrhotic livers and most frequently involve the right lobe. The tumors are typically soft, vary in color from gray green-yellow to light brown, are occasionally bile-stained, and often contain foci of hemorrhage or necrosis. The tumors can be single or multiple and range from less than 1 cm to more than 30 cm in diameter with a tendency toward larger sizes when involving non-cirrhotic livers. The traditional classification of Eggel[lxxvi] distinguishes three patterns of HCCs: multinodular, massive, and diffuse.

- Multinodular HCC typically is associated with cirrhosis
- In the massive pattern, a solitary tumor mass occupies much of the liver and may be associated with smaller satellite nodules. This pattern has been associated with noncirrhotic livers.
- The diffuse pattern is the least common and is characterized by numerous widespread small nodules that mimic cirrhotic nodules and virtually replace the entire liver.

In cirrhosis, clinically advanced liver disease has been associated with the diffuse or multinodular patterns of HCC. HCC may be pedunculated, presumably reflecting an origin within an accessory lobe. In more recent macroscopic classifications, HCCs are subdivided further into two main patterns based on growth characteristics. Expanding or expansive tumors have distinct borders that push aside the adjacent liver, and spreading or infiltrative tumors have poorly defined borders that microscopically invade the adjacent liver. Nodular HCC into an additional three subtypes:

- Type 1 is represented by HCC presenting as a single nodule
- Type 2 is a single nodule with extranodular growth
- Type 3 has a contiguous multinodular growth pattern

Microscopic Pathology

Hepatocellular carcinoma is classified histologically into well-differentiated, moderately differentiated, and undifferentiated (pleomorphic) forms.[lxxvii]

Well-Differentiated Appearance

Most of the tumors are well differentiated and it has 2 varieties, trabecular and acinar (pseudoglandular). In the *trabecular* variety, the malignant hepatocytes grow in irregular anastomosing plates separated by often inconspicuous sinusoids lined by flat cells resembling Kupffer cells. The malignant hepatocytes are polygonal, with abundant, slightly granular cytoplasm that is less eosinophilic than that of normal hepatocytes. Bile production is the hallmark of hepatocellular carcinoma, regardless of the pattern. Gland-like structures are present in the *acinar* variety.

Moderately Differentiated Appearance

Solid, scirrhous, and clear cell varieties of hepatocellular carcinoma are described. In the solid variety, the cells usually are small, although they vary considerably in shape. Pleomorphic multinucleated giant cells occasionally are present. Evidence of bile secretion is rare, and connective tissue is inconspicuous. In the scirrhous variety, the malignant hepatocytes grow in narrow bundles separated by abundant fibrous stroma. In most tumors, the cells resemble hepatocytes. More often, tumors contain areas of clear cells. The appearance of these cells results from a high glycogen or fat content.

Undifferentiated Appearance

The cells are pleomorphic varying greatly in size and shape. The nuclei also are extremely variable. Large numbers of bizarre-looking giant cells are present. The cells may be spindle-shaped, resembling those of sarcomas.

Fibrolamellar HCC

Fibrolamellar HCC, also known as oncocytic HCC or polygonal cell type HCC with fibrous stroma. This subtype is rare in Asia and showed male predominance. The lesions most often are large and solitary but may be multiple. The fibrous component often forms a central scar that can be demonstrated by radiological techniques. The neoplastic cells are larger than normal hepatocytes. They are polygonal in shape and possess granular, eosinophilic cytoplasm, a so-called “oncocytic” appearance, resulting from numerous swollen mitochondria. Pure fibrolamellar HCC has a better prognosis than ordinary HCC because it often presents as a surgically resectable lesion, and the fibrous component is thought to result in a slower rate of tumor growth. The fibrolamellar variant typically occurs in young patients with equal gender distribution. It does not secrete alpha-fetoprotein, is not caused by chronic hepatitis B or C and almost always arises in a noncirrhotic liver.[lxxviii]

Fine Needle Aspiration (FNA) of HCC

With regard to HCC, FNA is accurate with a sensitivity rate of 80 to 95% and a specificity rate of 100%.[lxxix] The sensitivity of guided FNA for diagnosing hepatic malignancy in most recent series is 90% to 96%, with a specificity of 90% to 100%. False-negative diagnoses of HCC are related either to very well differentiated tumors that are difficult to identify on the basis of cytology as being neoplastic or to poorly differentiated tumors that are difficult to distinguish as hepatocellular in origin.

The presence of at least two of three criteria (polygonal cells with centrally placed nuclei, malignant cells separated by sinusoidal endothelial cells and bile) was considered by Bottles et al[lxxx] to be 97% sensitive and 100% specific for HCC compared with other malignancies. Classic HCC is usually graded into well, moderately or poorly differentiated lesions. Histologic patterns comprise trabecular-sinusoidal, pseudoacinar and solid types; combinations are frequent.

Cytological Features

- Hepatocytic characteristics include polygonal cells with well-defined borders, ample granular cytoplasm, central round nucleus, well-delineated nuclear membrane, prominent nucleolus and fine, irregularly granular chromatin. Mitoses increase with nuclear grade.

- Cohesive clusters of malignant hepatocytes with arborizing, tongue-like projections of broad cords (>2 cells thick) that may be wrapped by peripheral endothelium.

- Well differentiated HCC cells tend to be conspicuous by their small size, monotony, subtle increase in N/C ratio and nuclear crowding. Poorly differentiated HCC cells tend

to be pleomorphic.

- Atypical naked hepatocytic nuclei are seen. Bile may be present within tumor cells or in canaliculi or pseudoacini.

- Intracytoplasmic fat and glycogen vacuoles are common. Intracytoplasmic inclusions include hyaline, pale and Mallory bodies. Intranuclear cytoplasmic inclusions are seen.

Treatment of HCC

The management of HCC involves multiple disciplines including hepatology, surgery, diagnostic and interventional radiology, oncology, and pathology. Treatment depends on severity of underlying liver disease, tumor bulk, associated comorbidities and availability of expertise in surgical resection, transplantation and ablative therapies.

Staging of HCC and prognosis

A precise staging of the disease may help decide on prognosis as well as choice of therapy with the greatest survival potential. There are several prognostic scoring systems including Barcelona-Clinic Liver Cancer (BCLC)[lxxxi], Cancer of the Liver Italian Program (CLIP)[lxxxii], the Chinese University Prognostic Index (CUPI) and Japanese Integrated Staging (JIS).

Okuda staging[lxxxiii]			
	Negative	Positive	Stage
Tumor size	<50%	>50%	I: No positive factors
Ascites	Absent	Present	II: 1-2 positive factors
Bilirubin	<3 mg/dl	>3 mg/dl	III: 3-4 positive factors
Serum albumin	>3 g/dl	<3 g/dl	

Strategy for staging and treatment assignment in patients diagnosed with HCC according to the BCLC proposal (Fig 2, Table 5)

The BCLC staging and prognostic system accounts for variables related to tumor stage, physical and liver functional status, and cancer-related symptoms and also provides a link to a treatment algorithm. Patients in stage A can undergo resection, transplantation, or ablation.

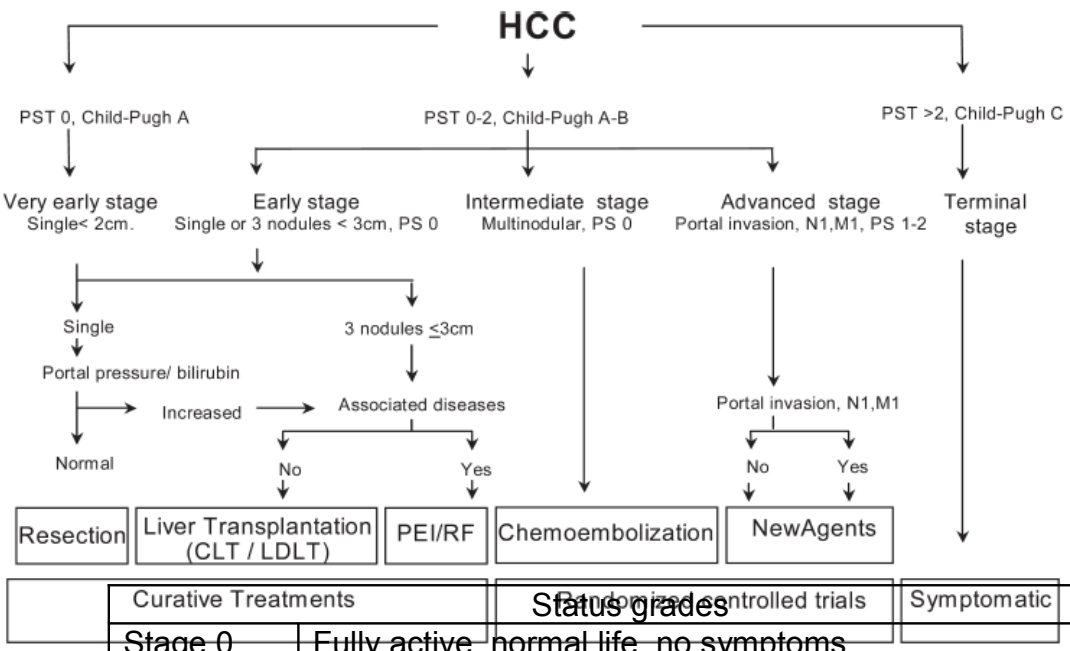


Figure 2

BCLC staging

Table 5 World Health Organization Performance Stage

Status grades	
Stage 0	Fully active, normal life, no symptoms
Stage 1	Minor symptoms, able to do light activity
Stage 2	Capable of self-care but unable to carry out work activities.Up for more than 50% waking hours
Stage 3	Limited self care capacity. Confined to bed or chair >50% waking hours
Stage 4	Completely disabled. Confined to bed or chair

CLIP Stage				
Points	CTP	Tumor morphology	AFP	Portal vein thrombosis
0	A	Uninodular ≤ 50% of liver	<400 ng/ml	No
1	B	Multinodular ≤50% of liver	≥400 ng/ml	Yes
2	C	Massive >50% of liver		

The Okuda classification takes into account radiologic tumor size and liver function (ascites, total serum bilirubin, and serum albumin) is helpful in identifying patients with advanced HCC but may be less adequate for staging patients with early or intermediate stage disease. Another commonly used staging system is the Cancer of the Liver Italian Program,[lxxxiv] which uses a mathematical score based on the CTP, tumor morphology, AFP, and presence of vascular invasion; however, it does not assess populations undergoing radical therapies, such as resection or transplantation. Multivariate analysis showed that the JIS, CLIP and modified CLIP scores were better staging systems for predicting survival than the Japanese and AJCC TNM. Best discrimination ability for patient survival was observed in JIS score and CLIP score.[lxxxv]

Table 6 Summary of Therapeutic Modalities for HCC and Their Outcomes

Treatment	Survival	Special issues
Surgical resection	1 y: 97% 3 y: 84% 5 y: 26%–57%	Choice of therapy for patients without cirrhosis (low morbidity) 5%–15% of HCC patients eligible Right hepatectomy has higher risk than left hepatectomy Pre/postresection adjunct therapy not recommended
Transplantation (LT)	1 y: 91% 2 y: 75% 5 y (MILAN): >70% 5 y (extended): >50%	Curative treatment for chronic disease and HCC MELD exception points for HCC Effective corresponding to UNOS criteria (1 tumor >5 cm; up to 3 tumors <3 cm Liver donor LT considered for HCC progression outside MILAN criteria UCSF criteria not implemented in current MELD exception allocation policy
Radiofrequency ablation (RFA)	1 y: 90% 3 y: 74% 5 y: 40%–50%	Effect is more predictable in all tumor sizes than following PEI Superior to PEI in larger tumors;

		equivalent in small tumors Requires fewer treatment sessions
Percutaneous ethanol injection (PEI)	1 y: 85% 3 y: 50% 5 y: 40%–50%	Early HCC patients not suitable to resection or OLT or RFA not available or contraindicated Highly effective for small HCC (<2 cm) Low rate of AEs
Transarterial Chemoembolization (TACE)	1 y: 82% 2 y: 63%	Nonsurgical patients with large/multifocal HCC w/o vascular invasion or extrahepatic spread

Surgical resection:

Only about 5% of the patients with HCC in Western countries and nearly 40% of patients in Asian populations are candidates for surgical resection. Resection is the treatment of choice for noncirrhotic patients who have HCC. The 5-year survival rate after resection can exceed 50%.[lxxxvi] [lxxxvii] Patient selection is the most important to achieve a long-term response. Those who have Child's class A cirrhosis, normal bilirubin levels, no portal hypertension (hepatic venous pressure measurements >10 mm Hg), and no varices, the 5-year survival rate reaches 70%.[lxxxviii] The presence of single tumors smaller than 5 cm is a favorable factor for resection because the risk of vascular invasion and dissemination decreases. The most important predictors of recurrence are microvascular invasion and multinodular tumors. Recurrence rates of 50%at 3 years and 70% at 5 years are seen after resection, but these rates are significantly lower when the favorable characteristics are present.[85]

Local ablation

Those who are at early stage HCC and not suitable for resection or OLT are the best candidates for local ablation. Ablation may be achieved by chemical (100% ethanol or 50% acetic acid) or physical (radiofrequency, cryoablation, or microwave) techniques. The efficacy

of percutaneous ablation is assessed by CT or MRI 1 month after the procedure and is indicated by the absence of contrast within the tumor. The recurrence rate is as high as that for resection.[85] Percutaneous ethanol injection is the most widely used ablation approach with the necrosis rate for HCC smaller than 2 cm is 90% to 100% but is reduced to 70% in tumors between 2 cm and 3 cm and is 50% in tumors between 3 cm and 5 cm. [lxxxix] [xc] A long-term study showed that with successful tumor necrosis the survival rate in Child's class A disease may reach 50% at 5 years.[xci] The potential drawback of ethanol ablation is the need for repeated sessions (average of four per patient) to achieve complete necrosis.

An alternative ablative technique is radiofrequency ablation. It involves the insertion of single or multiple electrodes that deliver heat around the tip leading a wide region of necrosis. The efficacy of radiofrequency ablation is similar to that of ethanol ablation for tumors smaller than 2 cm, but the technique requires only one session instead of four. The efficacy for tumors larger than 2 cm is better than that of ethanol ablation.[xcii] [xciii] Importantly, radiofrequency ablation has been shown to provide better local control of disease and is associated with better survival than ethanol ablation. The one potential drawback of radiofrequency ablation is a higher rate of adverse events such as pleural effusion, pain, and peritoneal bleeding. It is the ablative procedure of choice in most centers.

Transarterial chemoembolization

HCC is a highly vascular tumor that derives most its blood supply from the hepatic artery, whereas the rest of the liver is perfused by both the hepatic artery and the portal vein. Hence selective intra-arterial administration of chemotherapeutic agents followed by embolization of the major tumor artery has been performed to treat HCC. This procedure may be complicated by liver failure, possibly by the ischemic infarct of adjacent non-tumorous liver. There was a survival benefit observed in a meta-analysis with TACE versus control but no

survival benefit with embolization alone versus control. A recent randomized study compared TACE versus supportive care in 112 patients who had unresectable HCC[xciv]. Two thirds of the patients had multinodular tumors with a maximal tumor diameter of about 5 cm. Only TACE showed a survival benefit compared with conservative treatment (hazard ratio, 0.47; 95% confidence interval, 0.25–0.91). The tumor-free survival rate at 2 years was 63% for TACE and 27% for conservative management. These studies showed that TACE can lead to a survival benefit in carefully selected patients who have compensated or mildly decompensated liver function (78% in Child's class A patients and 22% in Child's class B patients), absence of tumor-related symptoms, renal failure, or portal vein invasion, and a maximal tumor diameter of about 5 cm.

Systemic chemotherapy/radiation/hormonal therapy

A variety of chemotherapeutic regimens have been used for patients who have HCC not amenable to any of the treatments discussed above. The results have been dismal and may be related in part to the limitations in choice of chemotherapeutic agents and the dose that can be used in patients who have underlying cirrhosis. Focal liver radiation targeted to the tumor has been shown to result in complete responses in 17% to 92% of unresectable HCC but is most effective in smaller lesions. About one third of patients develop radiation-induced liver damage, which may lead to hepatic decompensation.[xcv] There have been no randomized, controlled trials comparing radiation against local ablation or chemotherapy.

A new form of radiation therapy, direct intratumoral injection of Yttrium-90 spheres (TheraSphere) has been reported to result in complete destruction of nonresectable HCC.[xcvi] This technique needs to be evaluated further in randomized clinical trials as to whether it improves overall survival. Tamoxifen, chemotherapy, anti-androgens, and octreotide do not have efficacy or improve survival for HCC.

Liver transplantation

OLT is the best treatment option for HCC because it eliminates the tumor together with the entire diseased liver, thereby eliminating the risk for development of de novo HCC. In the early 1990s the results of OLT for HCC were dismal, with 1-year survival rates of 10% to 70% and 3-year recurrence rates up to 69%.[xcvii] Mazzaferro and colleagues published a landmark paper on OLT for HCC.[xcviii] When OLT was restricted to patients who had a single tumor of 5 cm or less and no more than three tumors, each less than 3 cm in diameter, the 4-year survival rate was 75%, and the recurrence-free survival rate was 83%. For the 35 patients (73%) who met the predefined criteria, the overall and recurrence-free survival rates were 85% and 92%, respectively. In the 13 patients (27%) who had tumors exceeding the criteria, the overall survival rate was 50%, and the recurrence-free survival rate was 59%. The United Network for Organ Sharing (UNOS) has adopted these criteria. The number of available donors worldwide and therefore only a finite number of transplantations for HCC will be performed. In addition, waiting time for liver transplants is increasing worldwide, from 6 months to more than a year. Therefore, some patients will not be able to proceed to OLT because of tumor progression or deterioration in medical condition. A recent study from Barcelona showed a decrease in survival rate from 84% to 54% as the waiting time to OLT increased from 62 to 162 days.[xcix] In the United States, patients who have HCC receive higher-ranking scores to shorten their waiting time and to prioritize them. The impact of the adjusted score on the post transplantation survival of patients who have HCC and the wait-list mortality of patients who have non-HCC end-stage liver disease remains to be determined.

The size limitations described by Mazzaferro et al[95] have been challenged. The group in San Francisco reported 1- and 5-year survival rates of 90% and 75%, respectively. Twenty-five percent of these patients had solitary tumors 5 cm to 6.5 cm in diameter or fewer than

three tumor nodules, each smaller than 4.5 cm and with a total diameter less than 8 cm.[c] These data were based on the size of the tumor at the time of explants examination not on size at diagnosis when the decision to transplant is planned. The authors suggested loosening the criteria of OLT for HCC, but these results should be confirmed by other centers before changing the current criteria. Live-donor transplant potentially can eliminate the significant waiting time and allow the surgery to be performed electively. A recent case series from Japan described 56 patients who underwent live-donor transplantation for HCC.[ci] The 1- and 3-year survival rates were 73% and 55% respectively, and six tumor recurrences were noted. The authors concluded that the 3-year survival rate was lower than that in patients who underwent live donor transplantation for nonmalignant liver disease (73%). However no data available the expansion of the criteria determined by Mazzaferro[95] for those receiving live-donor transplants for HCC.

Molecular therapies in HCC

Most of the treatments aim to abrogate signaling pathways related to proliferation and cell survival. Alternatively, other treatments rely on the blockade of growth factors and signals related to dissemination of the disease (e.g. angiogenesis, telomerase activation) etc. Most of the agents currently under investigation block membranous tyrosine kinase receptors (TKRs). The ligands for these receptors include EGF, PDGF, VEGF, and HGF. (Table 7)

Table 7 Molecular targeted therapies assessed in clinical trials in HCC[cii]

Treatment	Type molecule (target)
Sorafenib	Small molecule (TKI) RAF, VEGF, PDGFR
Erlotinib	Small molecule (TKI) EGFR inhibitor (TKI)
Cetuximab	Monoclonal antibody (Mab) EGFR inhibitor
Lapatinib	Small molecule (TKI) EGFR, Her2/nu

Sunitinib	Small molecule PDGFR, VEGFR, KIT (KI)
Bevacizumab	Monoclonal antibodies VEGF (Ab)

Sorafenib

Sorafenib is an oral multikinase inhibitor with activity against several tyrosine kinase (VEGFR2, PDGFR, c-Kit receptors), and serine/threonine kinases (b-Raf). This drug targets two of the main pathways involved in hepatocarcinogenesis by blocking angiogenesis (VEFGR2 and PDGFR) and cell proliferation through activation of Ras/MAPKK signaling (bRAF).[ciii] Sorafenib increases progression free survival in renal cancer and has recently been approved for use in the management of this cancer.

The randomized phase III double-blind placebo-controlled clinical trial[civ] conducted in patients with advanced HCC treated with sorafenib has shown improvement in survival of 3 months in patients with advanced HCC, which was not only statistically significant. The median overall survival was 10.7 months with sorafenib and 7.9 months with placebo (hazard ratio for death, 0.69; 95% confidence interval, 0.55–0.87; $p < 0.001$). Median time to progression was 5.5 months with sorafenib vs. 2.8 months with placebo (hazard ratio 0.58; 95% confidence interval 0.45–0.74; $p < 0.001$). Recently, the drug has been approved both by the FDA and EMEA for the treatment of HCC. Sorafenib is the first systemic therapy to prolong survival in HCC and, consequently, is the new reference standard treatment of patients with advanced HCC.

End of Literature Review

Materials and Methods

This descriptive study was carried out in the Department of Medical Gastroenterology, Madras Medical College, Chennai. This is the major referral tertiary care center available to the population of Tamilnadu, Pondicherry and neighboring states Andhra Pradesh and Karnataka. The main aim of our study was to assess the clinical and laboratory parameters in patients with hepatocellular carcinoma. The study was carried between the periods of February 2007 to January 2009. (24 months)

Inclusion Criteria

- Patients who are attending Department of Medical Gastroenterology with clinical diagnosis of Hepatocellular carcinoma. Cases were included if they met EASL diagnostic criteria for HCC.[67]

Diagnostic criteria for HCC

Cyto-histological criteria
Non-invasive criteria (restricted to cirrhotic patients)
1. *Radiological criteria: two coincident imaging techniques** Focal lesion >2 cm with arterial hypervascularization
2. *Combined criteria: one imaging technique associated with AFP* Focal lesion >2 cm with arterial hypervascularization AFP levels >400 ng/ml

Exclusion Criteria

- Patients who have been diagnosed as
  1. Hemangioma of liver
  2. Secondaries liver

3. Benign focal liver disorders

II. Study protocol

At the time of admission a detailed history was obtained from the patients or care giver. Their details entered in a preformed proforma. (Annexure) They were enquired about presenting complaints, the duration of illness, past h/o jaundice, blood transfusion. Detailed history of alcoholism and status of chronic liver disease and their treatment details obtained.

Patients were carefully examined at the time of admission. Various clinical parameters studied which include jaundice, pallor, stigmata of chronic liver disease. Enlarged liver was looked for size, surface, border and bruit. Splenomegaly, free fluid and neurological status were assessed. Stage of the liver disease assessed based on Child-Turcotte-Pugh Classification.

Child-Turcotte-Pugh Classification[cv]

Parameter	Numerical Score		
	1	2	3
Ascites	None	Slight	Moderate/severe
Encephalopathy	None	Slight/moderate	Moderate/severe
Bilirubin (mg/dL)	<2.0	2-3	>3.0
Albumin (g/dL)	>3.5	2.8-3.5	<2.8
Prothrombin time (seconds increased)	1-3	4-6	>6.0

The clinical stage and the severity of the disease were assessed based on Okuda stage and CLIP score.[82]

Okuda staging[83]			
	Negative	Positive	Stage
Tumor size	<50%	>50%	I: No positive factors
Ascites	Absent	Present	II: 1-2 positive factors
Bilirubin	<3 mg/dl	>3 mg/dl	III: 3-4 positive factors
Serum albumin	>3 g/dl	<3 g/dl	

CLIP Stage[82]				
Points	CTP	Tumor morphology	AFP	Portal vein thrombosis
0	A	Uninodular ≤ 50% of liver	<400 ng/ml	No
1	B	Multinodular ≤50% of liver	≥400 ng/ml	Yes
2	C	Massive >50% of liver		

All the patients enrolled in the study were investigated as outlined in the Proforma (Annexure). Patients were subjected for radiological investigations include ultrasound abdomen, Contrast enhanced Computed tomography (CECT) abdomen or MRI, whichever is feasible. Blood samples were collected for viral markers such as HbsAg, anti-HCV antibody, HIV serology, Liver function tests, and serum alpha fetoprotein (AFP). Ascetic fluid collected for biochemical, cytological investigations. Upper GI endoscopy was done to assess the grade of varices and any evidence of portal hypertension. After admission, once clinical diagnosis was made appropriate therapy was instituted. USG guided Fine needle aspiration and trucut biopsy was performed whenever is feasible and if not contraindicated.

Contraindications for FNAC/ trucut biopsy

1. Impaired hemostasis with prothrombin time more than 3 seconds over control, PTT more than 20 seconds over control, thrombocytopenia and markedly prolonged bleeding time

2. Severe anemia (Hb <8 g/dL) /Septic cholangitis/ Possible hemangioma/ Possible echinococcal (hydatid) cyst

3. Local infection near needle entry site, such as right sided pleural effusion or empyema, right lower lobe pneumonia, local cellulitis, infected ascites or peritonitis

4. Tense ascites (low yield technically, risk of leakage). High-grade extrahepatic biliary

obstruction with jaundice (increased risk of bile peritonitis)

5. Uncooperative patient/ Poor performance status

Patient preparation

Procedures and risks of the procedure were explained and informed consent was obtained. All aspirin products and non steroidal agents were discontinued at least 5 days beforehand. Injection vitamin K was given in jaundiced and liver failure patients. The patients were kept in empty stomach after midnight, the day prior to the procedure. Screening laboratory studies include CBC, PT/PTT, BUN, bleeding time, coagulation time and typing and cross matching for possible transfusion.

EQUIPMENT: Disposable automated Trucut biopsy gun –18 Gauge needle with 2 cm throw length, designed to cut out cores of tissue. Specimens obtained with this needle were less fragmented, even in the cirrhotic liver and thus a high success rate. Specimen was obtained using suction/aspiration into a 10 ml syringe. Trucut needle is a modernized Vim-Silverman needle.

Technique

Patient was laid supine in bed with right hand behind his head. Liver margins were estimated by ultrasound. Two approaches are popular, transthoracic (intercostal) or subcostal (anterior). With the former, biopsy site is identified along the midaxillary line in the center of hepatic dullness; usually the eighth or ninth intercostal space. This approach avoids other abdominal organs but always penetrates the pleura. With the subcostal approach, the biopsy site lies below the costal margin and is used when a liver mass is easily palpable below the right costal margin. The risk of visceral laceration is higher and this approach is infrequently used.

A wide area was prepped and draped in sterile fashion. The skin was anaesthetized with 1% lidocaine, and then deeper structures such as subcutaneous tissue, intercostal muscles and diaphragm were infiltrated in that order. A small superficial incision was made with a No 11 blade at the needle entry site to facilitate needle insertion. The first needle pass should sample the centre of the lesion since this will reduce contamination by cells from surrounding normal liver. The centres of large lesions may occasionally be necrotic and hence may not render diagnostic material. If the first pass yielded only necrotic debris and/or inflammatory cells, the second pass should be made close to the edge but well within the target. Under US guidance, an outer guide needle of larger diameter and 10 cm long was first introduced through the superficial layers. This outer needle will not only ensure needle stability, but will also allow multiple passes of the needle without inconvenience to the patient. The fine needle of 20 gauge was attached to a disposable syringe and was passed through it. When the tip of the fine needle was correctly located within the lesion by US, negative pressure was applied and the needle advanced steadily for 1-2 cm and moved back and forth. With the needle still in position negative pressure was released and needle withdrawn. The patient was asked to suspend respiration during advancement of the needle. Usually several passes of the needle were performed in slightly different directions to ensure representative sampling. The material in the needle was expelled on to glass slides and smeared immediately.

Through the outer needle, 18 gauge automated biopsy gun of 2 cm throw length was inserted and patient asked to suspend respiration. The position of the stylet was confirmed by US and then the device was fired. A 2.5 cm core of liver was aspirated and needle withdrawn. Several passes of the biopsy needle (2-3) were performed to minimize sampling bias.

Specimen: At least two to three liver cores, each more than 2 cm in length was routinely fixed

in 10% buffered formalin, specimen processed and the tissues stained with hematoxylin and eosin.Cytological preparation - fluid from aspirating syringe was smeared on clean microscope slides and sent to Cytology Laboratory. Smears were air-dried and stained with May-Grunwald-Giemsa as well as fixed in 95% alcohol and stained by hematoxylin and eosin.

Aftercare

Patients were monitored in a recovery area with frequent examination of vital signs (blood pressure, pulse) post biopsy. If no complications were apparent, they were transferred back to ward in stretcher. Strict bed rest was enforced for 24 hours. For the first 2 hours, patient was positioned on his right side. Vital signs were checked frequently. Diet was restricted to clear liquids for several hours, then full liquids as tolerated. Acetaminophen was usually sufficient for pain control. All patients signed informed consent prior to aspiration and the study protocol conformed to the ethical guidelines of the Declaration of Government General Hospital, as reflected in a prior approval by the Hospital's Human Research Committee. Patients were followed with serum alpha fetoprotein and radiological methods.

Statistical methods:

The quantitative data were presented as mean±SD or median (range). The continuous variables between the two groups were compared by the Mann–Whitney U-test. The Kruskal–Wallis test was used to compare a continuous variable across different stages of tumors. The P-value<0.05 was considered significant.

Results

Baseline characteristics

Seventy two patients who fulfilled the study criteria were included in the present study. Table 1 reveals that the mean age was 54 years with male female ratio of 3:1. (Figure 2) Asymptomatic hepatocellular carcinoma (HCC) observed only in 2 cases. Preadmission mean duration of illness was 54 days. Twenty two percent of patients had cirrhosis, of which only 7 were known cases of cirrhotics at admission.

Age distribution

The age distribution among the study population is summarized in Fig 1. Nearly half of the patients belong to the age group of 60 to 70 years. Nearly 15 percent of patients either belong to 30 -40 years or more than 70 years of age.

Clinical features at presentation

Among the symptoms abdominal pain and weight loss was most commonly observed symptoms. (Table 2, Figure 3 & 4) More than half of the patients had anorexia and or weight loss. The features of hepatic decompensation were seen in half the patients at first presentation with ascites in 56.9%, jaundice in 22.2% and hepatic encephalopathy in 4.2% of patients. Gastrointestinal bleed melena was present only in 1.4% patients. Hepatomegaly was seen in more than half the patients (81.9%) while in 18% of cases liver was not enlarged. The enlarged liver was hard in two-third cases and was firm in consistency in the remaining one-third. Clubbing, hepatic bruit and were less common. Abdominal lump as a presentation observed in 12.5% of patients.

Table 1 Baseline characteristics of patients with HCC

Parameters	**N = 72**
Age (years)	
Median (range)	55 (30–75)
Mean± SD	54±12
Mode	60
Sex (M : F)	54:18 (3:1)
Symptomatic HCC (%)	97.2
Incidental HCC (%)	2.8
Symptom duration before HCC diagnosis (days)	
Median (range)	30 (3–240)
Mean±SD	54±50
<1 month (%)	40.2
>1 month (%)	59.8
Known cirrhotic at diagnosis—no. (%)	7 (9.7)
Underlying cirrhosis at diagnosis—no. (%)	16 (22.2)

Table 2 Clinical profile of HCC

Symptoms	**(%)**	**Physical signs**	**(%)**
Abdominal pain	66.7	Hepatomegaly	81.9
Anorexia	56.9	Pallor	61
Weight loss	66.7	Edema	37.5
Jaundice	22.2	Icterus	25
Fever	6.9	Palpable spleen	22.2
Encephalopathy	4.2	Hepatic bruit	18.1
GI Bleed	1.4	Clubbing	2.8
Ascites	56.9	Abdominal lump	12.5

Figure 1 Age Distribution

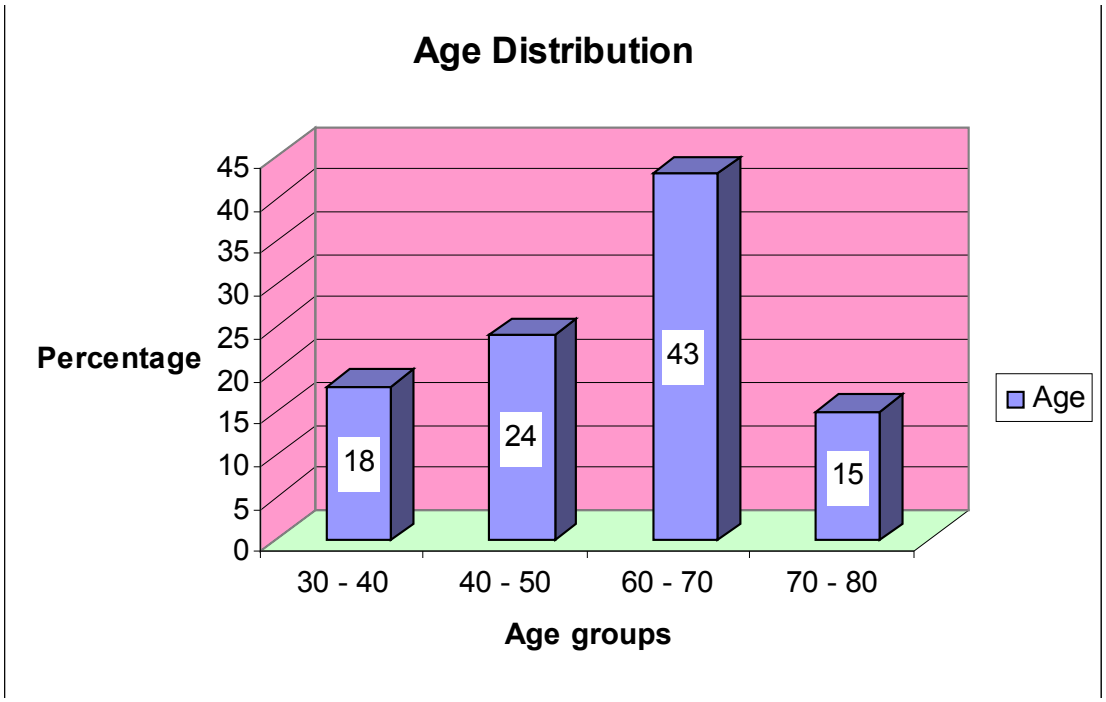


Figure 2

Sex

Distribution

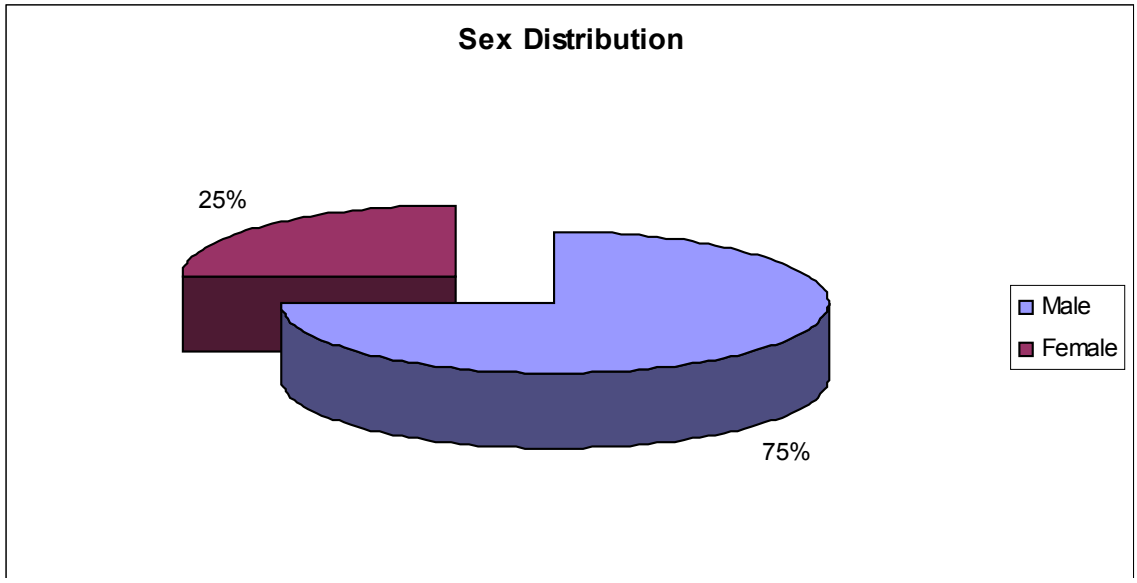
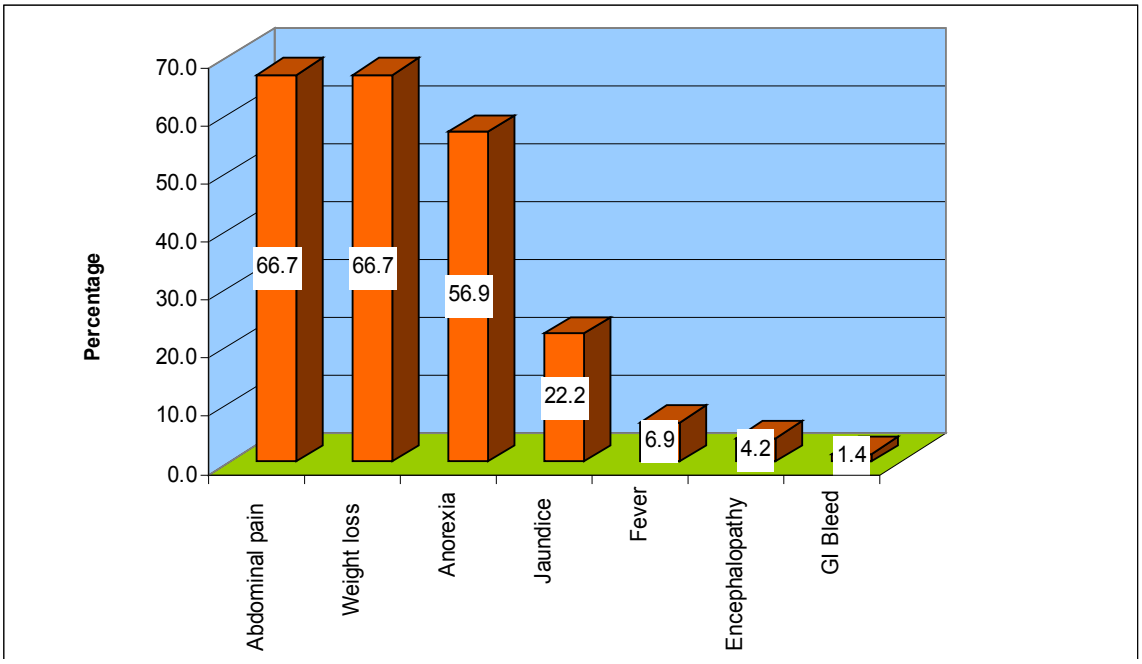
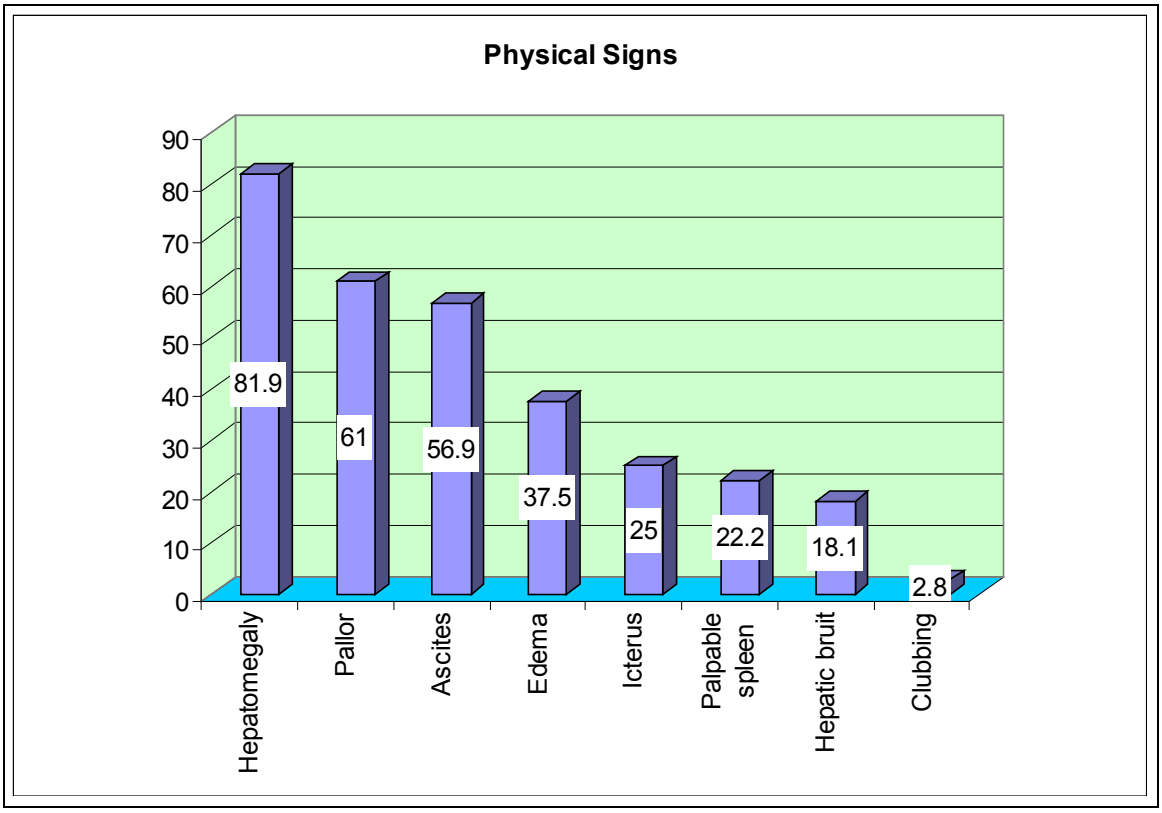


Figure 3 Symptoms of HCC

Figure 4 Physical Signs





Hematological, biochemical and endoscopic profile

The biochemical investigations were mildly deranged. Serum alpha fetoprotein (AFP) was diagnostic (>400 ng/ml) in 43 of 70 (61.5%) patients; with normal AFP in 14 of 70 (18.5%) patients. The median serum AFP value was 515 ng/ml (range 1.3–92625) (Table 3). Mean AST and ALT values were more than two time of upper limit of normal. The mean serum bilirubin was 2.6±3. More than three fourth of cases had esophageal varices with gastric varices of 8.3%. Nearly three fourth of cases had low serum albumin level of <2.8 gm %.

Etiological studies

The etiologic work up was available only in 52 (72%) patients, while in rest of 20 patients (27.7%) etiology was unknown due to incomplete investigation reports. HBV was the most common viral etiologic agent associated with HCC, observed in 23 of 52 (31.9%) patients either alone or as cofactor with alcohol or HCV (Table 4). Alcohol or HCV alone as an etiological agent was observed in 20.8% and 9.7% of cases respectively. Dual infection was observed only in one patient along with alcohol abuse. (Table 4)

Radiological studies

Radiological profile was analyzed from both US and CT scan regarding number, size, distribution and characteristics of liver lesions along with vascular details and metastases. (Table 5) Contrast enhanced CT scan abdomen was available for 61 of 72 cases. The HCC most commonly noted in the right lobe of liver (48.6%), followed by bilobar involvement

(34.7%). The left lobe was involved in approximately one fifth of cases. Multicentric HCC involving either one lobe or both lobes were seen in 43% of patients.

Table 3 Hematological, biochemical and endoscopic profile of HCC

Parameter	Mean ±SD	Median (range)
Hemoglobin (g/dl)	10.2±1.9	10 (3-14)
ESR	35.1±28.8	29.50 (5-182)
Serum bilirubin (mg/dl)	2.6±3	1.6 (0.5-2.6)
Serum albumin (g/dl)		
<2.8 g/dl (%)	20.8	
2.8–3.5 g/dl (%)	40.3	
>3.5 g/dl (%)	38.9	
AST (IU/l)	96.8±78.4	80.5 (0.9-425)
ALT (IU/l)	92.5±78.9	61 (17-394)
SAP (IU/l)	280±291	201.5 (22-1898)
Serum AFP (ng/ml) (n=70)*	8980±18372	515 (1.3-92625)
<10 ng/ml	20	
10–400 ng/ml	18.5	
>400 ng/ml	61.5	
Upper GI endoscopy (n=48)*		
Esophageal varices (%)	79.2	
Gastric varices (%)	8.3	
Portal hypertensive Gastropathy (%)	12.5	

*Number of patients in whom the investigation was done.

Table 4 Etiological work up of HCC

Etiology	No (%)
HBV related (n=52)*	23 (31.9)
HCV related (n=50)*	7 (9.7)
Alcohol alone	15 (20.8)
HBV+HCV	1
Alcohol +HBV	6
Alcohol +HBV+HCV	1
Alcohol +HCV	4

*Number of patients in whom the investigation was done.

Very large tumors (>5 cm) were seen in two-third of cases. The average size of HCC was 6±4 cm. Small HCC (<2 cm lesion) was seen in only 6% of patients approximately. Single lesion was the most common presentation of HCC observed in two-third cases. Three or more lesions were seen in about one-fifth of cases. The hyperechoic (42.7%) or hypo echoic (39.2%) lesions were the most common appearance on US observed in 80% of the cases. Similarly, the CT appearance of HCC was hypo dense in 21.4%, mixed or heterogeneous density in 52.4% and hyper dense in 26.2% patients. Vascular invasion of either major branch of splenoportal axis was seen in one fifth of the patients. Main trunk of portal vein or its main branches were involved in 20.8% patients. Extrahepatic spread of tumor was seen in 6 patients. Metastases involving retroperitoneal lymph nodes were seen in 3 of 6 patients (50%) followed by lung and pleural and left supraclavicular lymph node metastases in one each.

Histopathological study

Fine needle aspiration (FNA) and liver biopsy was available in 36 patients. Diagnosis of HCC was made based on cytohistology in 31 (86.1%) patients. In five patients (13.8%) cytohistology was normal. Nearly three fourth had well differentiated histology. No major complication or fatality was reported in any of these patients except mild pain at the site of needle puncture in few. (Table 6, Figures 5, 6,7,8)

Table 5. Tumor characteristics of hepatocellular carcinoma patients

Characteristics	Patient number (%)
Localization of tumor	
Right lobe	35 (48.6)
Left lobe	12 (16.7)
Both lobes	25 (34.7)
Single tumor	41 (56.9)
Multiple tumors	31 (43.1)
Tumor size	
<3 cm	11 (15.3)
3–5 cm	15 (20.8)
>5 cm	45 (62.5)
US appearance of HCC (n=71)	
Mixed-echo or heterogeneous	12 (17.7)
Hypoechoic	28 (39.2)
Hyperechoic	30 (42.7)
Isoecho	1 (1.4)
CT appearance of HCC (n=61)*	
Hypodense	13 (21.4)
Mixeddensity or heterogeneous	32 (52.4)
Hyperdense	16 (26.2)
Portal vein thrombosis	
No portal vein thrombosis	57 (79.2)
Main portal vein thrombosis	14 (19.4)
Right portal vein thrombosis	1 (1.4)

Table 6 Histopathological profile of HCC (n=36)*

	N=36 (%)
Total no Cytology & histopathology positive cases	31 (86.1)
Well differentiated	25 (69.4)
Poorly differentiated	6 (16.6)

*Number of patients in whom the investigation was done

Figure 5 Hepatocellular Carcinoma –FNA
Showing malignant hepatocytes with Endothelial Rimming (100x)

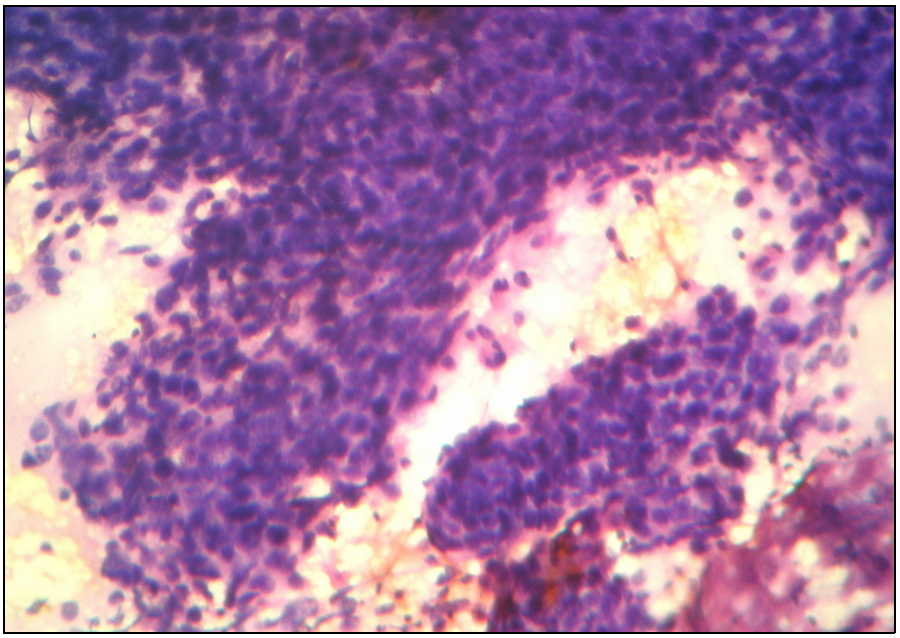
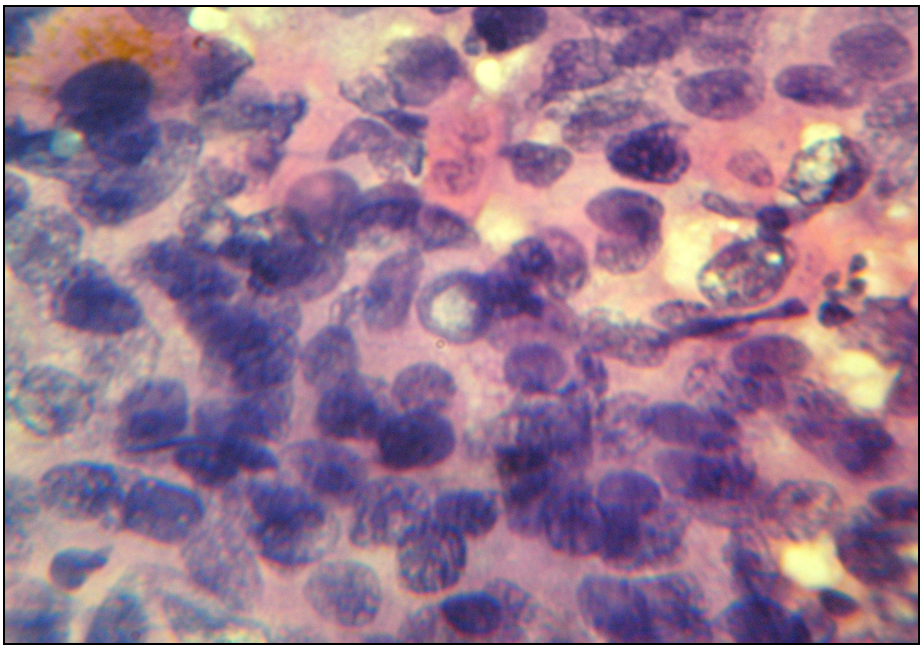


Figure 6 Hepatocellular Carcinoma –FNA
Showing Nuclear Pleomorphism & intranuclear inclusions (400x)



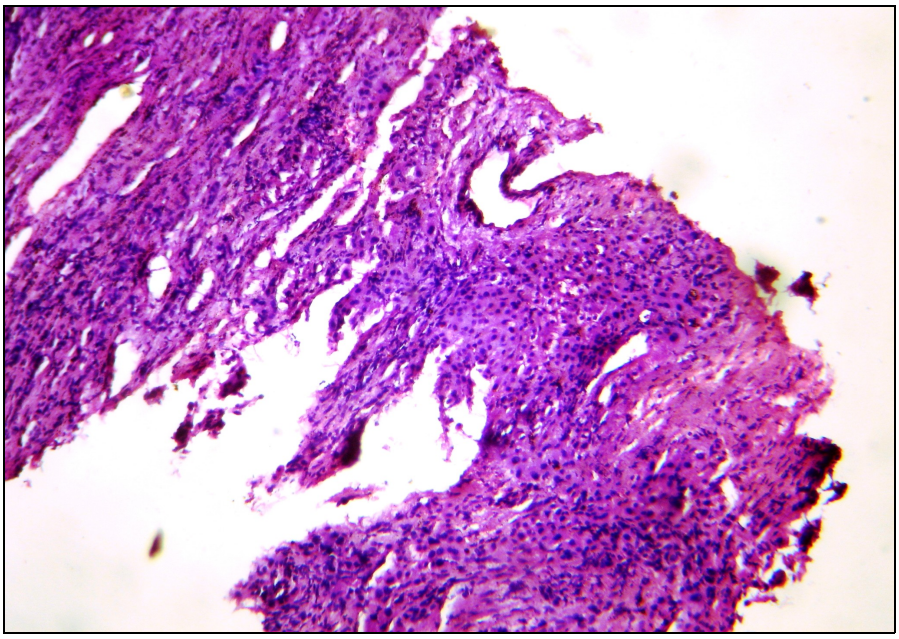


Figure 7 Hepatocellular Carcinoma-HPE (100x)

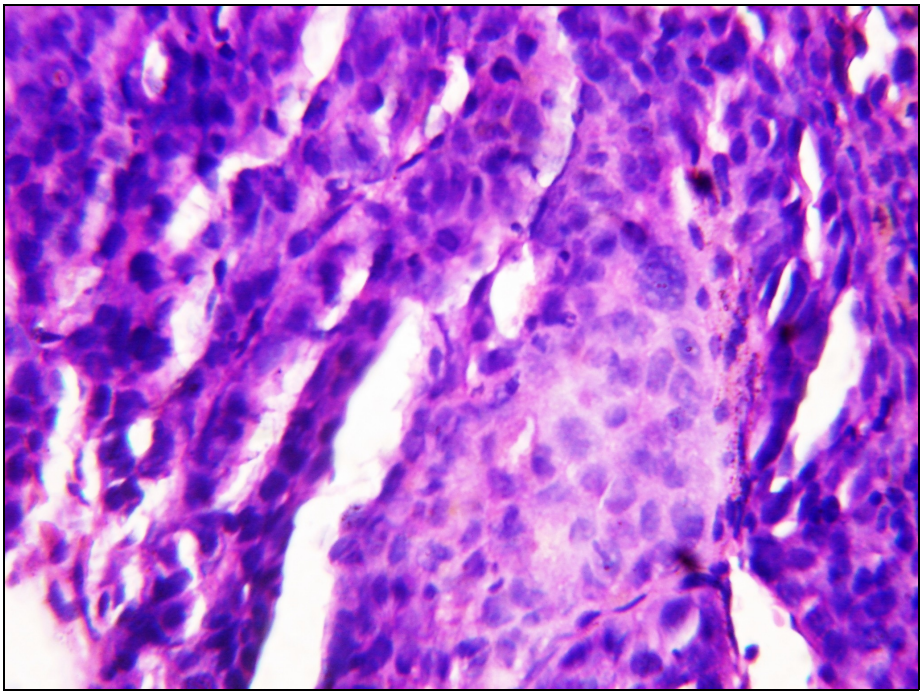


Figure 8 Hepatocellular Carcinoma-HPE (400x) Showing trabecular pattern and polygonal hepatocytes with increased pleomorphic hyperchormatic nuclei

Figure 9 CECT Abdomen showed contrast enhanced lesion noted in the left of the liver

Figure 10 MRI abdomen showed marked hepatomegaly with large fat containing heterogeneous hypervascular mass in segment 5 & 8

Staging of HCC

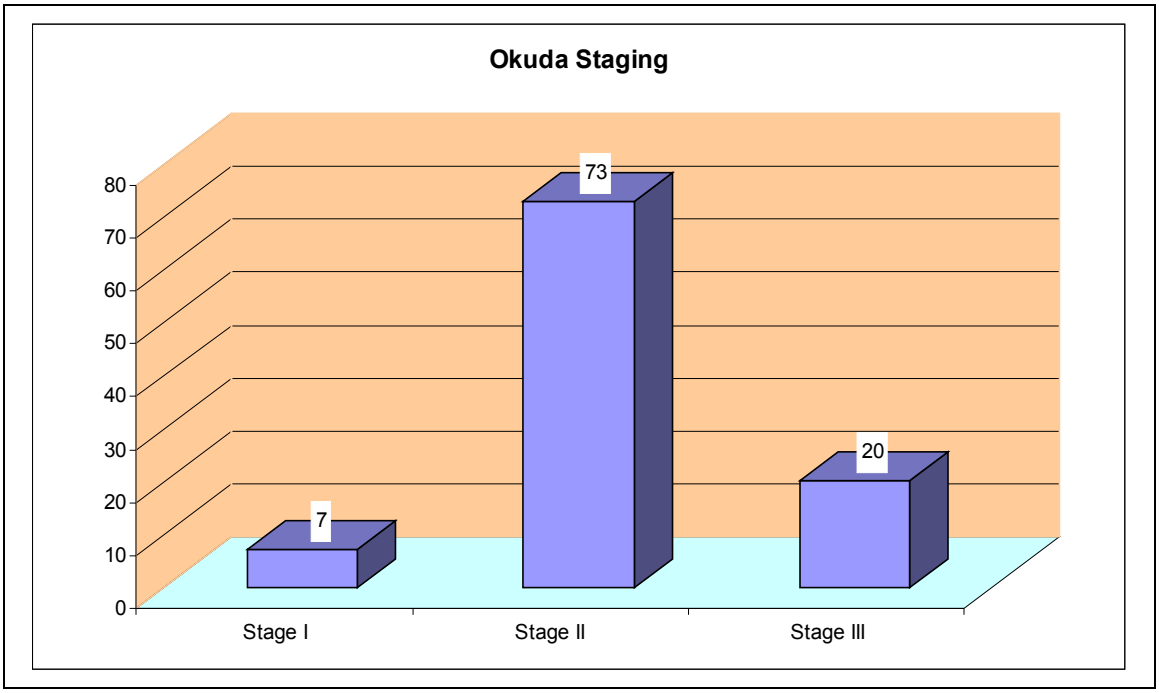
Based on clinical, radiological and laboratory investigations both Okuda staging and CLIP score was done. Okuda staging was possible in only 70 patients. More than three-fourth of HCC was in Okuda stage 2 (72.8%). Only five patients had Okuda stage 1 lesion. (Table 7, Figure 9) The size of HCC lesion was >5 cm in 73% of cases. Hence, HCC was large and very advanced in most of the cases (Table 6). More than ninety percent of patient had CLIP score of ≥2.The serum AFP was not related to different stages of Okuda and CLIP Score (P >0.05, NS).

Table 7 Okuda and CLIP staging of HCC and its correlation with serum AFP (n=70)

No (%)	AFP (ng/ml) Median (range)	P value
Okuda stage		>0.05*
I (n=5)	6 (2.9-60000)	
II (n=51)	515 (1.3-92625)	
III (n=14)	628.5 (7.3-50000)	
CLIP score		>0.05*
0 (n=7)	6 (2-54)	
1 (n=24)	515 (1.3-58452)	
2 (n=17)	727 (3.65-60000)	
3 (n=12)	497 (7.3-92625)	
4 (n=8)	10295.5 (1500-43000)	
5 (n=1)	420	
6 (n=1)	690	

* Statistically not significant.

Figure 9 Okuda staging



Discussion

Hepatocellular carcinoma (HCC) is one of the most frequent neoplasms worldwide causing increased morbidity and mortality. The incidence of HCC increases with age. The development of HCC is uncommon before 40 years of age in western world. However, the pattern of HCC incidence by age is sometimes dependent on the geographic pattern or on etiologic factors.[5]

The age distribution of patients with HCC in the present study was similar to other studies in past. Studies from India have shown the maximum incidence of HCC in the fifth to sixth decade.[cvi] [cvii] Our study showed that 43 percent of cases belong to 60 to 70 years of age. Only 15% belongs to 30 – 40 years age group. A very similar observation made by Saini et al.[34] In our study, HBV-related HCC occurs in older age group as compared to HCV-related HCC. This is in contrast to the earlier observations that HCV related HCC observed in older age groups, i.e. at sixth decade. [8, 9]

HCC commonly observed in male sex. The male preponderance is similar to other studies.[12, cviii] The population-based data show a male to female ratio of 3:1–2:1.1.[cix] In our study showed male female ration of 3:1. High preponderance of HCC in males in our study could be due to gender bias in seeking medical treatment. And this could be partially explained by the fact that men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores.[4] Hospital based data from various studies made similar observation.[35]

The overall clinical presentation of HCC patients in our study was similar to other studies.[34, 35] Asymptomatic HCC observed only in two of our patients. Low incidence of asymptomatic HCC detection possibly could be due to referral bias or possibly due to non availability of screening programs for the high risk groups. In a series of 461 Italian patients,

asymptomatic HCC was detected in 23%.[63] Hepatic decompensation as presentation observed in 56% of our patients. New-onset ascites, recurrent variceal hemorrhage, or progressive encephalopathy should always raise suspicion for HCC. Abdominal pain was one of the most frequent manifestations in patients with HCC is reported in 66.7% of our patients. Pain abdomen was reported as a feature in 46% of Japanese[115] and 90%–95% of African[114] patients with HCC. Acute onset pain may also reflect intraperitoneal bleeding, may present as an acute abdomen — this is one of the most dramatic and life threatening presentations of HCC. This type of presentation not observed in any of our cases. In sub-Saharan Africa and Southeast Asia, spontaneous rupture of HCC is the most common cause of spontaneous hemoperitoneum, with an incidence of approximately 10% of patients with HCC.[cx] Less acute bleeding is much more common in patients with advanced HCC; and at the time of autopsy, more than 50% of patients have blood-stained ascites.[cxi] A similar presentation of hemorrhagic ascites noted in 11.1% of our cases, of which, 2 had only hemorrhagic ascites as a major clinical presentation.

The incidence of gastrointestinal bleed was 1.4% in the present series. Kumar et al reported 20% of their cases presented with varices bleed, whereas the other series quoted the incidence of 2-7.6%.[114, 115] Yeo *et al*[cxii] reported that varices represent the source of bleeding in only half of the HCC patients presenting with gastrointestinal bleeding; in those patients with non-variceal bleeding, duodenal ulceration is the most common cause.

Jaundice represents an important clinical presentation of HCC was observed in 22.2% of our cases, but reported incidence vary from 5% to 44% in earlier studies.[cxiii] Jaundice complicating HCC has been classified into one of two types.[112] The hepatocellular type (90%), related to extensive tumor infiltration of a cirrhotic liver. These patients have a grim prognosis, with 90% of them dying within 10 weeks of their first clinical presentation. Other rare causes of jaundice include reactivation of the underlying viral hepatitis, and alcohol- or drug-induced

hepatitis. Patients with type 1 obstructive jaundice have an intraluminal obstruction by a tumor thrombus. Biliary obstruction due to clot formation secondary to hemobilia of the biliary tree is typical of a type 2 obstruction. Type 3 obstruction is characterized by extraluminal neoplastic compression of the biliary tree. The paraneoplastic syndrome was not reported in our case series. Kumar et al[35] reported only one patient developed troublesome hypoglycemia. The incidence of paraneoplastic syndromes especially hypoglycemia has been reported in up to 30% cases. The rarity of paraneoplastic syndrome has been seen in other Indian series as well.[12, 106]

Underlying cirrhosis was seen in 31.9% of our HCC patients. HCC with underlying cirrhosis reported in 70–86% of Indian autopsy series and the clinical studies observed 30–80% of cases.[11, 12] The present study is in conformity with the previous studies from India.[12, 34] However, some cases of cirrhosis might have been missed in the present study, as evidence of cirrhosis may not have been strictly looked for in all cases.

Frequency of Clinical Features of HCC

Clinical data	South Africa[cxiv]	Japan [cxv]	Italy[63]	India [35]	Current series
Abdominal pain	95	46.2	38	68	66.7
Anorexia	25	44.7	6.7	74	56.9
Weight loss	34	28.9	8	55	66.7
Ascites	51	26.5	17.5	51	56.9
Fever	35	16.7	12	36	6.9
Jaundice	28	16.7	14	35	22.2
Variceal bleeding	2	7.6	4	22	1.4
Hepatomegaly	-	-	90	84	81.9
Palpable mass	92	23.3	-	-	12.5
Ankle edema	-	16.8	-	37.5	37.5
Asymptomatic	-	-	38	-	2.8

The HBV is the most common etiologic factor in Asian countries. It accounts for up to three-fourth cases of HCC, while HCV infection may account for 10–15% of HCC cases.[cxvi cxvii]

[cxviii] HbsAg positivity in our cases was 31.9%, which is comparable with other Indian studies (36% to 74 %).[10, 11, 31] The prevalence of anti-HCV antibody in Indian population varies from 0.3% to 1.8%.[cxix] In our case series HCV positivity noted only in 9.7% of cases. However, PCR-based studies have found HCV RNA positivity in 27–33% of patients with HCC. Serological evidence of HCV infection in patients with HCC in India is 15%.[12] Dual infection with or without alcohol was seen in 2% of patients, similar lower incidence observed in previous studies.[12, 35]

India, despite being an intermediate endemic zone for HBV has low incidence of HCC unlike other Asian countries. This phenomenon is akin to low HCC incidence in Greenland Eskimos as compared to Alaskan Eskimos despite similar HBsAg positivity.[cxx] However, in a proportion of patients the etiology was unknown due to incomplete work up or absence of markers for either HBV or HCV and alcohol. This is because of as patients may not have been aggressively investigated, once an advanced lesion was detected ruling out curative therapy in most. An earlier prospective study, the patients with unknown etiology constituted almost 20% of patients despite aggressive work up including for p53 gene mutation and dietary contamination with aflatoxin B.[12] Such a high proportion of HCC patients without a known etiologic factor may point to the possibility of another hitherto unknown factor or pathogenetic mechanism for HCC in India and needs further study.

Sensitivity of AFP ranges from 39% to 64%, specificity 76–91% and positive predictive value 9–32%.[69, cxxi] These figures possibly suggest that the serum AFP estimation may not a good screening tool for HCC. As per the diagnostic criteria adopted by EASL, serum AFP >400 ng/ml was present in only 61.5% cases. More than two third (61.5%) of our patients had elevated AFP values beyond diagnostic level. Others had either normal level (20%) or levels were in non-diagnostic range (18.5%). There are many studies available showing that, serum AFP is frequently elevated in patients with liver cirrhosis without HCC and can be normal or

only moderately elevated in patients with hepatocellular carcinoma.[cxxii] The median AFP level was only 515 ng/ml in the study population despite advanced HCC was noted in three-fourth cases. There are some studies which suggest that the production of AFP depends on the size or the degree of differentiation of the hepatoma cells.[cxxiii] AFP values were high among histopathologically proven HCCs compared to negative HCCs, and it is statistically significant. ($p<0.02$). AFP showed no association with size of the tumor ($p>0.05$). AFP values were high among HbsAg positive HCCs ($p<0.05$), whereas no difference among anti HCV tested patients. When compared according to either CLIP score or Okuda staging there was no difference in the serum AFP level to differentiate between early and advanced lesion.

Like previous studies most of the lesions on US were hypo echoic (39.2%) or hyperechoic (42.7%) in appearance. However, vascular invasion observed in one fifth of cases (20.8%), one-third (34.7%) having bilobar distribution and 6.9% had distant metastases. Even when lesions were single they were large enough in most of the cases to rule out curative resection. Another study from a tertiary care center in India showed that 56% of patients with HCC had tumor size larger than 5cm and high incidence of vascular invasion (main portal vein in 43% and right portal vein in 30%) with very low resection rate.[34] Right lobe of the liver (48.6%) was involved in majority as has been reported in other studies. More than half (56.9%) of the patients had a single tumor. Tumor was >5 cm in the majority (62.5%) of the patients. Moreover portal vein was thrombosed in 15 (20.8%) patients.

Cytohistopathological examinations, including fine needle aspirations and biopsies were undertaken in 36 patients without complication. Both FNA and biopsy was done for the diagnosis of HCC once a lesion was suspected on radiological investigation. The cytohistological examination was positive in 86.1% of patients and was falsely negative in only 6.4% cases. A similar observation noted by Kumar et al.[35] It was safe and easy to perform. The larger and advanced lesions might have contributed to better results with FNA in

the present study. FNA appears to be the best diagnostic modality as it has low false negative rate as compared to serum AFP, once a lesion is detectable on CT scan or US. In a country like ours, a costly investigation like serum AFP with such low sensitivity should be used if cytohistological examination is equivocal in patients with radiological suspicion of HCC.[35] However, in an otherwise unapparent HCC on radiological investigation, screening with serum AFP is the only practical modality available now, despite its limitations.[35] Cytohistological diagnosis is recommended only if atypical vascular pattern observed radiological observed in cirrhotic patients. [29]

Almost two-third of patients with HCC had CLIP score of more than 2 and Okuda stage 2 and 3. The Okuda staging and CLIP score of HCC revealed a very high proportion of advanced lesions in this study with expected low survival. We could follow up only 8 of our patients, with survival varying from 5 months to 24 months. In view of these findings, we need to evolve a better screening and surveillance program for early detection of HCC in patients with chronic liver disease in our country.

Summary and Conclusion

1. The study includes total of seventy two patients, mean age was 54 years with male female ratio of 3:1.
2. Nearly half of the patients belong to the age group of 60 to 70 years.
3. Mean preadmission duration of illness was 54 days.
4. Twenty two percent of patients had cirrhosis and asymptomatic hepatocellular carcinoma (HCC) observed only in 2 cases.
5. Among the symptoms abdominal pain (66.7%) and weight loss (66.7%) were most commonly observed symptoms.
6. Hepatic decompensation was seen in half the patients at first presentation with ascites in 56.9%, jaundice in 22.2% and hepatic encephalopathy in 4.2% of patients.
7. Abdominal lump as a presentation observed in 12.5% of patients.
8. Hemorrhagic ascites noted in 11.1% of our cases
9. The median serum AFP value of 515 ng/ml (range 1.3–92625) observed in the study population. Diagnostic value of AFP >400 ng/ml was present in only 61.5% cases with normal AFP in 14 of 70 (18.5%) patients.
10. More than three fourth of cases had esophageal varies with gastric varices of 8.3%.
11. HBV was the most common viral etiologic agent associated with HCC, observed in 23 of 52 (31.9%). Alcohol or HCV alone as an etiological agent was observed in 20.8% and 9.7% of cases respectively
12. The average size of HCC was 6±4 cm (mean±SD). Very large tumors (>5 cm) were seen in two-third of cases.
13. CT appearance of HCC was hypo dense in 21.4%, mixed or heterogeneous density in 52.4% and hyper dense in 26.2% patients.

14. Vascular invasion of either major branch of splenoportal axis was seen in one fifth of the patients.
15. Diagnosis of HCC was made based on cytohistology in 31/36 (86.1%) patients.
16. More than three-fourth of HCC was in Okuda stage 2 (72.8%). Only five patients had Okuda stage 1 lesion. More than ninety percent of patient had CLIP score of ≥2. The serum AFP level was not associated with different stages of Okuda and CLIP Score (P >0.05, NS).

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