Hepatocellular carcinoma: Epidemiology, risk factors and pathogenesis

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Hepatocellular carcinoma (HCC) is the commonest primary malignant cancer of the liver in the world. Given that the burden of chronic liver disease is expected to rise owing to increasing rates of alcoholism, hepatitis B and C prevalence and obesity-related fatty liver disease, it is expected that the incidence of HCC will also increase in the foreseeable future. This article summarizes the international epidemiology, the risk factors and the pathogenesis of HCC, including the roles of viral hepatitis, toxins, such as alcohol and aflatoxin, and insulin resistance.
Although currently relatively low, the incidence of HCC is rising in developed western countries[9-12]. In the United States, there has been an increase of about 80% in the annual incidence of HCC during the past two decades. HCC rates increased from 1.4/100 000 per year from 1976-1980 to 2.4/100 000 per year from 1991-1995[3,10]. This increase has been most marked in men, with African-American men having higher incidence rates than US Caucasian men. This was explained by the emergence of hepatitis C during this same period, although the rise in immigration from HBV-endemic countries may also have played a role[9,10].

Other developed western countries have noted similar increasing trends. An increase in incidence of HCC has been reported in Italy, the United Kingdom, Canada, Japan, and Australia. The increase was reported among immigrants from parts of the world with high prevalence, such as sub-Saharan Africa and parts of Asia, being associated with a parallel increase in hospitalization and mortality for HCC[3,10]. In Egypt, between 1993 and 2002, there was an almost twofold increase in HCC amongst chronic liver patients[10].

However, it is not obvious when this rising trend, observed in many countries, will reach a peak. The Disease Control Center in Atlanta has estimated that deaths related to chronic hepatitis C in the United States will triple from the current rates of 8-10 000 per year during the next decade. While most of these deaths will be due to liver failure and its complications, a considerable proportion can be expected to be due to HCC as well[9].

The major risk factor for the development of HCC is cirrhosis of the liver. However, about one quarter of HCC cases diagnosed in the United States do not have any known predisposing risk factors. The major known risk factors for HCC are viral (chronic hepatitis B and hepatitis C), toxic (alcohol and aflatoxins), metabolic (diabetes and non-alcoholic fatty liver disease, hereditary haemochromatosis) and immune-related (primary biliary cirrhosis and autoimmune hepatitis)[13]. Recently, the geographical variability in the incidence of HCC has been attributed to the changing distribution and the natural history of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection[28].

HCV

HCV is the most important risk factor for HCC in western European and North American countries, since epidemiological studies have shown up to 70% of patients with HCC have anti-HCV antibody in the serum[12,21-23]. Liver cancer has a higher prevalence in patients with HCV-associated cirrhosis than in non-viral aetiologies of chronic liver disease, while only a few cases of HCV-associated HCC have been reported in the non-cirrhotic liver, indicating that the virus possibly has a mutagenic effect[12,24,25].

The prevalence of HCV infection varies considerably by geographical region. African and Asian countries reported high HCV infection prevalence rates, while rates in North America, Europe and Australia have usually reported lower rates[26,27]. Egypt has the highest prevalence of HCV in the world[28,29] (predominantly genotype 4), which has been attributed to previous public health eradication schemes for schistosomiasis[28,30]. Even higher HCV infection rates, up to 60%, have been reported in older individuals, in rural areas such as the Nile delta, and in lower social classes[28,31,32,34].

The natural history of HCV infection has been investigated in several studies[8]. A Japanese study[35] reported a time lag of 13 years from infection by transfusion of
HCV infected blood to the development of chronic hepatitis. This time period was reported to be approximately 10 years in an American study and it took about 20 years for the same patients to develop cirrhosis of the liver[36]. Development of HCC took 28 years in the American subjects and 29 years in the Japanese cohort[35,36]. The annual risk of developing HCC in HCV-infected patients depends on the presence and severity of the underlying liver disease[39].

Up to 80% of HCV-infected individuals fail to eliminate the virus acutely and progress to chronic HCV infection[27-30,32]. Continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis is thought to lead to chromosomal damage and possibly to initiate hepatic carcinogenesis[27,30].

The rate of fibrotic progression following HCV infection is markedly variable, since the natural history of the disease typically extends over several decades[40,41]. The rate of fibrotic progression in HCV-infected patients is influenced by age at the time of infection, male sex, HCV genotype and alcohol consumption[42-45]. It is not clear whether any of these factors affect the onset of liver-related complications by mechanisms other than their effects on the rate of fibrotic progression. To determine which interactive variables were independent determinants of adverse clinical outcomes, Khan and colleagues examined the development of liver-related complications of chronic HCV in a large cohort of patients who were heterogeneous in age, country of birth, mode of HCV acquisition, HCV genotype, and histological and functional severity of liver disease. Patients were followed up for five years. These authors found that the major independent predictors of liver-related complications were sporadic transmission, advanced liver fibrosis at entry and low albumin[46].

**HBV**

The WHO has reported HBV to be second only to tobacco as a known human carcinogen[51]. Many studies on HCC risk following chronic HBV infection have been conducted in the East Asian countries, where most patients acquired HBV as newborn infants[52,53]. The incidence of HCC in HBV-related cirrhosis in this area of the world has been reported to be 2.7/100 000 children in 1981-1986, 0.57 in 1986-1990 and 0.70 per 100 000 children in 1981-1986, 0.57 in 1986-1990 and 0.36 in 1990-1994. A significant decrease in HCC incidence in adults was also observed, 3-4 decades later[52,53].

**HBV factors in HBV-related HCC**

The mechanisms of carcinogenesis in HBV infection have been extensively studied, and a major factor is chronic necroinflammation with subsequent fibrosis and hepatocyte proliferation. However, HCC may occur in HBsAg carriers without cirrhosis. Both HBV and host hepatocytes may contribute to the final pathogenic outcomes, either individually or synergistically. Therefore, it is reasonable to consider that apart from host factors, viral factors are likely involved in HBV-related hepatocarcinogenesis[54].

**Viral proteins in hepatocarcinogenesis**

HBV may encode oncopgenic viral proteins that may contribute to hepatocarcinogenesis[55]. For example, HBx is a well-known viral non-structural gene that has roles as a multifunctional regulator modulating gene transcription, as well as controlling cell responses to genotoxic stress, protein degradation, apoptosis, and several signalling pathways[56,57,58]. Although the specific mechanisms are still unknown, its critical role in liver malignant transformation has been demonstrated in studies of transgenic mice with HBx overexpression[59,60]. HBx protein has been shown to complex the tumor suppressor p53 protein and to suppress its function[61,62].

**HBV genotype, basal core promoter (BCP) mutation and viral load in hepatocarcinogenesis**

Several viral factors other than viral proteins as viral genotype, BCP mutations in the viral genome and viral load have been associated with hepatocarcinogenesis[63]. Eight HBV genotypes (A-H) have been described, based on genomic sequence divergence[64,65]. These have distinct geographical and ethnic distributions: genotypes A and D prevail in Africa, Europe, and India; genotypes B and C in Asia; genotype E only in West Africa; and genotype
F in Central and South America\textsuperscript{[20,79]. It is reported that HBV genotype affects clinical outcome and treatment responses. For example, in Asia, genotype C is found to be commonly associated with more severe liver disease, cirrhosis and the development of HCC, compared to genotype B\textsuperscript{[20,65,68,74-78] whereas in Western Europe and North America, genotype D is more associated with severe liver disease and a higher incidence of HCC, than genotype A\textsuperscript{[20,79]. In addition to viral genotype, specific viral genomic mutations, particularly the BCP T1762/A1764 mutation, also correlate with HCC risk\textsuperscript{[20,80,81].} }

A prospective cohort study with 11 years of follow-up assessed the relationship between HBV viral load and mortality. Viral load was found to be associated with increased mortality from HCC and chronic liver disease in HBV-infected subjects. The relative risk (RR) for HCC mortality in patients with viral load < 10\textsuperscript{5} copies/mL was 1.7 (95% CI, 0.5-5.7), whereas it was 11.2 (95% CI, 3.6-35.0) in patients with viral load > 10\textsuperscript{5} copies/mL\textsuperscript{[74-78]. Viral load may thus be a useful prognostic tool in HBV infection.

**HBV factors in young-onset HCC**

Viral factors in association with the development of HBV-related HCC in young patients seem to be different from their old-aged counterparts\textsuperscript{[20,82]. Tsai and colleagues compared serum viral loads in young (less than 40 years of age) and older (over 40 years) patient groups in 183 HBV-related HCC patients and 202 HBV carriers. These authors found high serum HBV DNA levels were associated with the development of HCC in older patients, rather than those under 40 years\textsuperscript{[20,83]. Another study from Taiwan demonstrated that genotype B was significantly more common in patients with HCC, aged under 50 years, compared to age-matched inactive carriers (80% vs 52%, \( P = 0.03\))\textsuperscript{[20,84].} This predominance was even more striking in younger patients with HCC, with 90% in those under 35 years. Most of these patients did not have cirrhosis. A further Taiwanese study reported that 26 children with HBV-related HCC were documented among 460 HBV carriers during 15 years follow up and genotype B was the major genotype (74%)\textsuperscript{[20,84]. These data suggest that genotype B-HBV may be associated with the development of HCC in young carriers without cirrhosis\textsuperscript{[20].}

**Viral factors in HCC in the absence of cirrhosis**

Studies of HBV-related HCC in patients without cirrhosis have helped to explain the effect of viral factors in HCC development. Liu et al.\textsuperscript{(2006) examined the role of BCP T1762/A1764 mutation, pre-core A1896 mutation and serum viral load in liver cancer, presenting in the absence of cirrhosis, by comparing 44 patients without cirrhosis, but with HBV-related HCC, to 42 individuals with cirrhosis and HBV-related HCC. These authors found that male gender, BCP T1762/A1764 mutation and viral load greater than 10\textsuperscript{5} copies/mL were independently associated with the risk of HCC development in the absence of cirrhosis. They suggested that viral features predisposing to HCC might be similar between cirrhotic and non-cirrhotic groups\textsuperscript{[20,85].}

**Pre-S deletion in HCC**

Recently, pre-S deletion of HBV has been found to be associated with the progression of liver disease and development of HCC in HBV carriers\textsuperscript{[20,86]. Pre-S deletion mutants hasten the storage of large envelope proteins in hepatocyte cytoplasm which can stimulate cellular promoters by inducing endoplasmic reticulum stress\textsuperscript{[53,67,68]}.}

The interactions between pre-S deletion, PC mutation and BCP mutation of various stages of chronic HBV infection were investigated in 46 chronic HBV carriers and 106 age-matched carriers with different stages of liver diseases; 38 with chronic hepatitis, 18 with cirrhosis, and 50 individuals with HCC\textsuperscript{[53]. Logistic regression analysis demonstrated that pre-S deletion and BCP mutation were significantly associated with the development of progressive liver disease. Combinations of mutations, especially the pre-S deletion, rather than single mutation were correlated with a greater risk of progressive liver disease. Sequencing analysis showed that the deleted regions were more common in the 3’ terminus of pre-S1 and the 5’ terminus of pre-S2\textsuperscript{[20,86]}.}

**Combined hepatitis B and hepatitis C**

Follow up studies have shown that patients with combined HCV and HBV infection have a higher risk of developing HCC than those with a HCV or HBV alone\textsuperscript{[53,69]. The cumulative risk of developing HCC was 10%, 21%, and 23%, respectively, after 5 years and 16%, 28% and 45%, respectively, after 10 years\textsuperscript{[53].}

The HCC risk in subjects with both infections was investigated in a meta-analysis of 32 epidemiological studies between 1993 and 1997\textsuperscript{[53,90]. The OR for development of HCC in HBsAg positive, anti-HCV/HCV RNA negative subjects was 20.4; in HBsAg negative, anti-HCV/HCV RNA positive subjects, 23.6; and subjects positive for both markers, the OR was 135. These data suggest a more than additive effect of HBV and HCV coinfection on HCC risk. The two viruses may possibly act through common, as well as different, pathways in the carcinogenic process. Given that HBV acts as a cofactor in the development of HCV related cirrhosis and HCC, vaccination of patients with chronic hepatitis C against HBV has been recommended aiming to avoid further liver injury\textsuperscript{[53,92,93].}

**Coinfection of HBV and hepatitis D virus (HDV)**

HDV coinfection with HBV is associated with increased liver damage. Verme and coworkers showed that HBsAg positive patients with HDV superinfection develop cirrhosis and HCC at an earlier stage (mean age 48 years), compared to HBsAg carriers without HDV infection (mean age 62 years)\textsuperscript{[53,91].}

**Coinfection with HIV**

Chronic hepatitis C is more aggressive in HIV positive subjects, leading to cirrhosis and liver failure in a shorter time period\textsuperscript{[53,90]. Coinfection with HIV is a frequent occurrence because of shared routes of transmission. A recent study of HCC in HIV-HCV coinfected patients indicated rapid development of HCC in these patients\textsuperscript{[53,96].}
Role of schistosomiasis

Schistosomiasis is a common parasitic infestation in some parts of the world. In Egypt, Schistosomiasis is a major public health problem and infection with Schistosoma mansoni constitutes the major cause of liver disease. From 1950s until 1980s, the Egyptian Ministry of Health (MOH) conducted a community-wide therapy campaign using parenteral tarter emetic to control the Schistosomiasis infestation. However, this unfortunately established a large reservoir of HBV infection in the country through needle re-usage at the time of treatment[97]. There is some epidemiological evidence that the presence of schistosomal infection may modify the course of hepatitis C genotype 4 co-infection and may lead to significantly more complications, such as portal hypertension at an earlier stage with accelerated progression to hepatitis C-associated fibrosis and thus quicker progression to HCC, than those patients who do not have a parasite burden[13,31-34].

Role of aflatoxin B1 (AFB1)

AFB1 is produced by a fungus of the genus, Aspergillus spp, in Asia and sub-Saharan Africa in which climatic factors and storage techniques favour the fungus to be a common contaminant of foods, such as grain, corn, peanuts and legumes. Areas with high exposure of AFB1 coincide with areas with a high prevalence of HCC. It has also been suggested that a high intake of AFB1 in HBV-infected patients is an added risk factor for HCC development[57,139,99,90]. It has been observed that areas with a high prevalence of HCC and high aflatoxin intake also correspond to areas with endemic HBV infection, and that patients at highest risk of developing HCC are those who are exposed to both HBV and AFB1[158].

Somatic mutations of the tumor suppressor p53 gene are the commonest genetic abnormality in human cancer and evidence supports a high level of p53 alterations in HCC. El Far and colleagues investigated p53 mutations in Egyptian patients with HCC and its relation to other prognostic factors, such as tumor grade, α-fetoprotein (AFP) and liver function tests to elucidate their implication in HCC pathogenesis. These authors found that p53 detection increased the frequency of HCC prediction from 79.5% to 86.3%. Moreover, significant positive correlation between p53 mutation and tumor size for tumor grade II and III was identified. Thus, serum concentration of p53 protein may be a potential non-invasive screening test for predicting risk of HCC[100].

It has been suggested that AFB1 can lead to HCC through inciting a specific mutation of codon 249 of the p53 tumor suppressor gene[101]. However, this mutation has also been found in patients who had previous contact with the HBV[102,103].

Pesticides

Pesticides exposure is one of the environmental factors hypothesized to increase the risk of HCC. Pesticides are considered to be possible epigenetic carcinogens through one or several mechanisms, such as spontaneous initiation of genetic changes, cytotoxicity with persistent cell proliferation, oxidative stress, inhibition of apoptosis, suppression of intracellular communication and construction of activated receptors[102,103].

A case-control study of HCC in HBV and/or HCV infected patients from Egypt suggested pesticides had an additive effect on the risk of HCC in rural males, amongst whom the use of carbamate and organophosphate compounds is commonplace[103].

Diabetes mellitus

A population-based study from the USA found diabetes to be an independent risk factor for HCC, regardless of chronic HCV or HBV infection, alcoholic liver disease, or non-specific cirrhosis. Diabetes was associated with a two- to threefold increase in HCC risk. About 60% of patients with HCC in this study were not diagnosed with chronic HCV-related or HBV-related hepatitis, alcoholic liver disease, or other known causes of chronic liver disease. Among these patients, 47% had diabetes, which was higher than those with other risk factors (41%). This suggests that diabetes may represent a considerable proportion of patients with idiopathic HCC[104].

An increased risk of HCC among patients with diabetes alone was also reported in a population based study using data obtained from the Denmark cancer registry[104,105]. Also, it is reported a threefold increased risk of liver cancer among patients hospitalized with diabetes, as well as and a fourfold risk in the presence of hepatitis, cirrhosis, and alcoholism in a Swedish study[104,106].

Diabetes, as part of the insulin resistance syndrome, has been implicated as a risk factor for non-alcoholic fatty liver disease (NAFLD), including in its most severe form, non-alcoholic steatohepatitis (NASH). NASH has been identified as a cause of both “cryptogenic cirrhosis” and HCC[104,107-112].

Diet

Many epidemiological studies have examined the relationship between diet and HCC risk[113]. The results are somewhat conflicting. Some studies have shown an inverse relationship between HCC and diets which are high in milk, wheat, vegetable, fish and fruit content. Other studies have shown no association.

With regard to egg consumption, two studies reported an inverse relation with HCC risk[114,115], while three others reported an increased risk[116,117,118]. Similarly, two studies[119,120] demonstrated that meat and animal protein consumption were associated with increased risk of HCC, although other studies[114,116] did not support this finding[119].

To verify if consumption of soya foods reduce the HCC risk, Sharp and his colleagues conducted a case-control study within a cohort of Japanese A-bomb survivors. They compared the pre-diagnosis intake of isoflavone-rich miso soup and tofu to HCC risk, adjusting for hepatitis B (HBV) and C (HCV) viral infections. They concluded that consumption of miso soup and other soya foods may reduce HCC risk and this is consistent with the results of epidemiological, animal and laboratory-based studies, as well as some clinical trials[120].
This is explained by the opposing effect of isoflavones on oestrogen and testosterone levels which reduce HCC risk, possibly by modifying the hormonal profile and reducing cell proliferation, associated with increased cancer risk. An alternative explanation may be that isoflavones provide an independent anti-tumour effect, such as suppression of angiogenesis, or stimulation of apoptosis.[12,126]

A 41% reduction in HCC risk among coffee drinkers, compared to non-drinkers, has been observed in a meta-analysis study.[121] This favourable effect of coffee drinking was established both in studies from southern Europe[122-124], where coffee is widely consumed, and from Japan[125,126], where coffee intake is less frequent, and in subjects with chronic liver disease.[127] Some compounds in coffee, including diterpenes, cafestol, and kahweol, may act as blocking agents via modulation of multiple enzymes involved in carcinogenic detoxification as demonstrated in animal models and cell culture systems.[119,127,128] Moreover, coffee components modify the xenotoxic metabolism via induction of glutathione-S-transferase and inhibition of N-acetyltransferase.[121,129] Other components of coffee, including caffeine and antioxidant substances from coffee beans, have been related to favorable modifications in liver enzymes such as γ-glutamyltransferase and aminotransferase activities.[119,127,128].

CONCLUSION

HCC is one of the commonest cancers worldwide. It is a major health problem and its incidence is increasing. The presence of cirrhosis is the major risk factor and worldwide this is largely due to chronic HCV and HBV infection. HCC carcinogenesis is likely to involve interplay of viral, environmental and host factors. The advent of mass-vaccination programmes for hepatitis B, particularly in East Asia is beginning to reduce prevalence rates for HCC in some countries, but for the most part, HCV-related HCC is increasing. Conquered strategies need to be developed for HCC surveillance in at risk populations.

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