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EDITORIAL

Non-viral causes of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and represents an international public health concern as one of the most deadly cancers worldwide. The main etiology of HCC is chronic infection with hepatitis B and hepatitis C viruses. However, there are other important factors that contribute to the international burden of HCC. Among these are obesity, diabetes, non-alcoholic steatohepatitis and dietary exposures. Emerging evidence suggests that the etiology of many cases of HCC is in fact multifactorial, encompassing infectious etiologies, comorbid conditions and environmental exposures. Clarification of relevant non-viral causes of HCC will aid in preventative efforts to curb the rising incidence of this disease.

Key words: Hepatocellular carcinoma; Etiology; Non-alcoholic steatohepatitis; Obesity; Diabetes; Alcohol; Tobacco; Oral contraceptive

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INTRODUCTION

Hepatocellular carcinoma (HCC) represents an international public health concern as one of the most common and deadly cancers worldwide. Globally, HCC accounts for 85%-90% of primary liver cancers[1] and its lethality is underscored by the fact that it is the third most frequent cause of cancer-related mortality[2]. In those patients who are not transplant candidates, HCC is particularly lethal, with a 5-year survival of less than 5%[3]. In the United States, the incidence of HCC appears to be increasing, with a more than twofold increase observed from 1976 to 2002 (Figure 1)[1,3,4]. A significant proportion of this increase is accounted for by the growing prevalence of hepatitis C virus (HCV) infection[5]. However, other potential causes of HCC are garnering close attention.

Increased body mass index and diabetes with subsequent development of non-alcoholic steatohepatitis (NASH) represent significant risk factors for HCC. This is especially concerning in light of the growing epidemic of obesity in adults and children over the past 25 years[1,5-8]. Other non-viral causes of HCC include iron overload syndromes, alcohol use, tobacco use, oral contraceptive use, aflatoxin exposure and betel quid chewing, a prevalent habit in the developing world. Emerging evidence suggests that the etiology of many cases of HCC is in fact multifactorial, including both viral infections and non-viral environmental and dietary exposures. This review focuses on the non-viral causes of HCC.

HEREDITARY HEMOCHROMATOSIS AND IRON OVERLOAD SYNDROMES

Hereditary hemochromatosis, a condition characterized by excess iron absorption, is caused by mutations in the

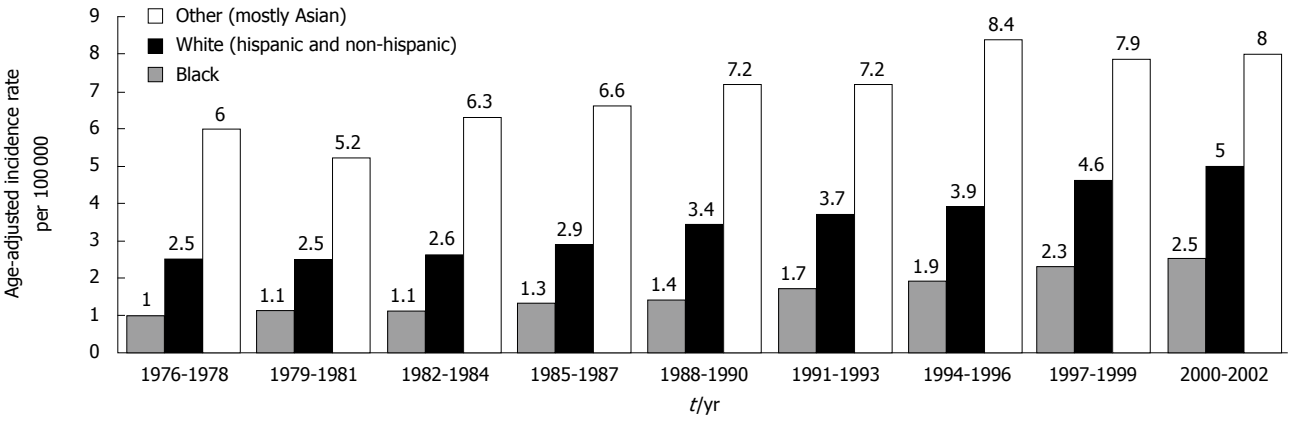

Figure 1 Average yearly, age-adjusted incidence rates for hepatocellular carcinoma men and women in the United States shown for 3-year intervals between 1976 and 2002. Whites included approximately 25% Hispanics, whereas other race was predominantly (88%) Asian. Source: Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) SEER*Stat Database: Incidence-SEER 13 Regs Public-Use, Nov 2004 Sub (1973-2002 varying), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2005, based on the November 2004 submission. Reprinted from El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; 132: 2557-2576, Copyright (2007), with permission from Elsevier[1].

HFE gene and/or other mutations in the iron metabolism machinery. This condition represents one of the most common autosomal recessive genetic disorders, affecting as many as 1 in 200 people of Northern European descent[9-11]. The *HFE* gene is required for efficient *in vivo* iron metabolism and two mutations within the *HFE* gene product, C282Y and H63D, have been well described in patients with hereditary hemochromatosis[10]. The C282Y mutation, which results in a base pair substitution in which tyrosine is substituted for cysteine at amino acid 282, is found in the homozygous state in up to 83% of patients with hereditary hemochromatosis[10]. The H63D mutation, characterized by substitution of histidine with aspartic acid at codon 63, is present in a minority of cases of hereditary hemochromatosis either in a homozygous state or with one copy of the C282Y mutation, a state referred to as a compound heterozygote[10]. The clinical significance of this latter mutation within the *HFE* gene, however, continues to be controversial.

The altered iron metabolism seen in hereditary hemochromatosis leads to excess iron storage in the liver and the subsequent development of liver dysfunction. Although other organs systems are also susceptible to iron overload, the liver bears the majority of malignant disease, with those patients with hereditary hemochromatosis being 20 times more likely to develop liver cancer than all other cancers combined[12].

Several population-based and case-control studies have shown that the diagnosis of hereditary hemochromatosis confers a consistent and markedly elevated risk for the development of HCC[12-17]. A sentinel study from the US National Center for Health Statistics found that patients who were diagnosed with hereditary hemochromatosis and who died were 23-fold more likely to have liver cancer compared to those without a diagnosis of hemochromatosis [Proportionate Mortality Ratio (PMR) 22.5, 95% CI: 20.6-24.6)[13]. In addition, the relationship between hereditary hemochromatosis and HCC is modified by diabetes, sex and genetics. Subjects with liver

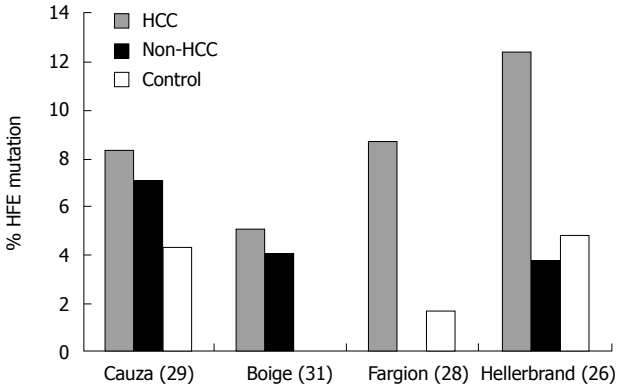

Figure 2 Prevalence of HFE mutations among patients with hepatocellular carcinoma, cirrhosis without hepatocellular carcinoma (non-hepatocellular carcinoma), and normal controls. Reprinted from Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. *Gastroenterology* 2004; 127: S79-S86, Copyright (2004), with permission from Elsevier [24]. HCC: Hepatocellular carcinoma.

cancer and concomitant diabetes mellitus were 82 times more likely to have a diagnosis of hemochromatosis[13]. Furthermore, a population-based study from Scandinavia found that men with hemochromatosis had a 29-fold increase in risk of liver cancer, whereas women with hemochromatosis had a sevenfold increase in risk[12]. Lastly, highlighting the genetic predisposition of disease and its consequences, an analysis of 5973 first degree relatives of patients with hemochromatosis found that these subjects had a nearly twofold increase in risk of HCC[12].

The presence of a single copy of the C282Y HFE gene mutation, although not diagnostic for hereditary hemochromatosis, has been studied to determine its prevalence and clinical significance in patients with HCC (Figure 2)[18-23]. Researchers comparing 81 patients with cirrhosis and HCC to 128 normal controls observed a significantly higher prevalence of the C282Y mutation in patients with HCC[18]. Another group observed that patients with HCC had a higher frequency of the C282Y mutation when compared to cirrhotic controls without HCC and healthy controls[19].

Additionally, they demonstrated that those subjects with the C282Y mutation had higher levels of serum ferritin, transferrin saturation, and hepatic iron deposition when compared to those without the C282Y mutation[19]. These studies suggest that increased iron load in HCC patients with a C282Y mutation exerts a cause and effect relationship in hepatocarcinogenesis[18,19]. This risk of HCC in patients with the C282Y mutation may not be equally conferred to all however. A recent prospective cohort study from France found that the C282Y mutation and iron overload were associated with a significantly increased risk of HCC among patients with alcoholic cirrhosis but not among those with HCV-related cirrhosis[23].

Contrary to the data presented above, two well-executed European studies did not find a significant difference in the prevalence of the C282Y mutation between patients with and without HCC[21,22]. Researchers from France observed comparable proportions of the C282Y heterozygous state in 133 cirrhotic patients with HCC and 100 without[21]. Likewise, in another cohort of 162 consecutive patients with HCC, the majority with cirrhosis, the frequency of the C282Y mutation did not differ from historical healthy controls or patients with HCV[22]. Concrete conclusions from these studies might be elusive, however, because of the small sample sizes, differences in the prevalence of the C282Y mutation in the respective populations, and referral bias to tertiary care centers[24].

More studies are therefore needed to determine correctly, in larger populations, the prevalence and effect of a single copy of the C282Y mutation. Additionally, on an individual basis, further study is needed to better characterize the comorbid, demographic and genetic factors that play a role in the risk of HCC in those with a single copy of the C282Y mutation.

It has also been proposed that the H63D mutation is not directly associated with hemochromatosis[10,25]. Certainly, none of the aforementioned studies observed a significant difference in the prevalence of the H63D mutation between patients with and without HCC[18-22]. Future studies are needed to assess further the relationship between this *HFE* gene mutation and the development of HCC.

Hereditary hemochromatosis is only one of the iron overload syndromes that leads to excessive iron deposition in the liver and other tissues. In fact, those patients with excess total body iron secondary to other etiologies have been shown to have a higher risk of HCC in the absence of genetic hemochromatosis[26-28]. Studies have suggested that conditions such as β thalassemia or iron overload in people of African descent might be associated with an increased risk of HCC[27,29,30]. One such study found that African iron loaded subjects had a 10-fold increase in the risk of developing HCC after adjusting for viral hepatitis, alcohol use and environmental exposures, such as aflatoxin[27]. Regardless of etiology, iron overload is not a benign condition and when recognized, surveillance for HCC should be undertaken.

NON-ALCOHOLIC FATTY LIVER DISEASE

Several case reports and subsequent observational studies have proposed that non-alcoholic fatty liver disease (NALFD), and more specifically, NASH, confers an elevated risk of developing HCC (Figure 3)[31]. NAFLD is a spectrum of clinical disease that ranges from benign or bland steatosis to NASH. The latter stage of this disease, through a process of chronic inflammation and subsequent hepatic fibrosis, can lead to cirrhosis[32]. The presence of cirrhosis itself is an independent risk factor for the development of HCC[33]. To characterize the natural history of NALFD, 420 patients identified in Olmstead County, MN, USA with the disorder were followed for an average of 7 years to determine overall mortality as well as liver related morbidity and mortality. In this population-based study, NAFLD was associated with a 34% increase in mortality and a significant increase in the risk of HCC, with two cases or 0.5% being diagnosed over the period of follow-up[34]. In subjects with NASH-related cirrhosis, however, the rate of HCC approached 10%[34]. These findings are well aligned with a series of studies from Japan. In one report, among 82 NASH patients treated from 1990 through 2001, six patients with HCC were identified over 11 years of follow-up[35]. All six patients developed HCC

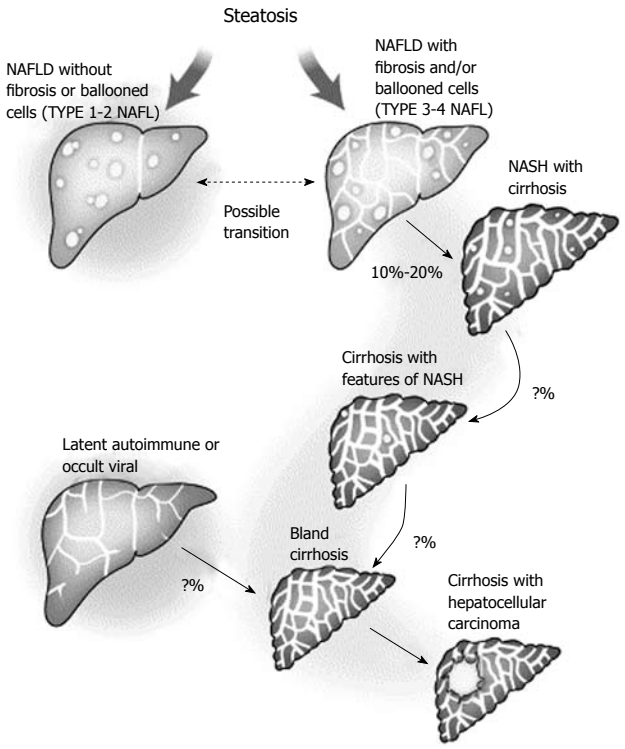

Figure 3 Progression of non-alcoholic fatty liver disease to cryptogenic cirrhosis. The explanation for the disappearance of steatosis remains uncertain but it is likely to be multifactorial and to involve changes in blood flow and exposure to fat-promoting hormones, as well as possible changes in the intracellular metabolism as a result of long-standing exposure to lipid peroxidation. Theoretically, this could represent a form of lipoatrophy that occurs within the fat-storing hepatocytes. Other forms of chronic liver disease may also present with a well-established bland cirrhosis. Efforts are needed to define better residual markers of past silent disease to improve our understanding of cryptogenic cirrhosis. Reprinted from Caldwell SH, Crespo DM. The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver disease. *J Hepatol* 2004; 40: 578-584, Copyright (2004), with permission from Elsevier[31]. NALFD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

Table 1 Characteristics of cohort studies included in the meta-analysis

Study	Country	No. of cases (men/women)	Study participants	Assessment of exposure	Adjustments
Møller *et al* (1994)	Denmark	22/36	Men: 14531 Women: 29434	Discharge diagnosis of obesity	Age
Wolk *et al* (2001)	Sweden	15/13	Men: 8165 Women: 19964	Discharge diagnosis of obesity	Age, calendar year
Nair *et al* (2002)	USA	659[1]	Men and women: 19271[1]	Measured	Age, sex, race, diabetes
Calle *et al* (2003)	USA	620/345	Men: 404576 Women: 495477	Self-reported	Age, race, education, marital status, smoking, physical activity, aspirin use, estrogen-replacement therapy (women), alcohol, dietary factors
Samanic *et al* (2004)	USA	322 whites/38 blacks	White men: 3668486 Black men: 832214	Discharge diagnosis of obesity	Age, calendar year
Kuriyama *et al* (2005)	Japan	69/31	Men: 12485 Women: 15054	Self-reported	Age, type of health insurance, smoking, intakes of alcohol, meat, fish, fruits, vegetables, bean-paste soup[2]
Batty *et al* (2005)	UK	51	Men: 18403	Measured	Age, employment grade, marital status, physical activity, smoking, other[3]
Oh *et al* (2005)	Korea	3347	Men: 781283	Measured	Age, area of residence, family history of cancer, smoking, exercise, alcohol
Rapp *et al* (2005)	Austria	57	Men: 67447	Measured	Age, occupational group, smoking
N'Kontchou *et al* (2006)	France	220[1]	Men and women: 771[1]	Measured	Age, sex, cirrhosis cause, diabetes
Samanic *et al* (2006)	Sweden	297	Men: 362552	Measured	Age, smoking

[1]Patients with cirrhosis; [2]ORs for women were further adjusted for age at menarche, age at end of first pregnancy, and menopausal status; [3]Other factors adjusted for include disease at entry, weight loss in the last year, height-adjusted FEV1, triceps skinfold thickness, blood pressure-lowering medication, blood pressure, plasma cholesterol, glucose intolerance, and diabetes. Reprinted by permission from Macmillan Publishers Ltd: [Br J Cancer]. Larsson SC, Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. *Br J Cancer* 2007; 97: 1005-1008, copyright (2007)[42].

in the setting of NASH-related cirrhosis[35]. In an update to this original observation, over a 17-year period, the authors found that among 382 patients with NASH, HCC was diagnosed in 34, 9% of the cohort, with 11 patients diagnosed during a 40-mo mean follow-up[36]. Comparing those NASH patients with and without HCC, multivariate logistic regression analysis identified older age (OR: 1.1, 95% CI: 1.03-1.2) and advanced hepatic fibrosis (OR: 4.2, 95% CI: 1.8-9.7) as independent predictors for the development of HCC[36]. In a prospective study of 118 patients with NASH and advanced liver fibrosis from the same cohort, the observed 5-year cumulative incidence of HCC was 7.6%, with HCC accounting for 46% of all fatalities[36]. In summary, these data highlight an association between NASH cirrhosis and an increase in the incidence of HCC over that of the general population. Therefore, regular HCC surveillance is imperative in patients with NASH cirrhosis.

The impact of NASH on the incidence of HCC may well be underestimated. In advanced fibrosis, an absence of steatosis may be appreciated, a finding which can obscure identification of the underlying etiology of liver injury in these patients. In this case, patients might be classified as having cryptogenic cirrhosis. In a United States study that examined 105 consecutive patients with HCC, after HCV, cryptogenic cirrhosis was the most common etiology of liver injury[37]. Among patients presenting with cryptogenic cirrhosis, 58% had a body mass index (BMI) $\geqslant$ 30, 47% had diabetes, and 50% had a prior histological diagnosis of NASH or clinical characteristics consistent with NAFLD. Furthermore, only 23% of subjects with cryptogenic cirrhosis were undergoing surveillance for HCC in comparison to 61% of subjects who had a history of HCV-related liver disease[37]. Clearly, these observations emphasize the importance of HCC surveillance in this group of patients and the failure thus far to appropriately screen for HCC in this disease process.

OBESITY

The prevalence of obesity has increased to epidemic proportions over the last three decades. Excess body mass is classified as overweight if the BMI is > 25 kg/m^2 and < 30 kg/m^2, or obese if the BMI is $\geqslant$ 30 kg/m^2. In addition to the increase in an array of disease processes observed with being overweight or obese, both classifications of excess body mass are associated with a higher risk of developing all cancers, including liver cancer[38]. In one population-based study from Sweden, 28 cases of HCC were diagnosed in 28129 patients from 1965 to 1993, thus conferring an almost threefold higher risk of HCC in obese patients[39]. A recent European case-control study observed a significantly increased risk of HCC among obese (OR: 3.5, 95% CI: 1.3-9.2) or diabetic (OR: 3.5, 95% CI: 1.6-7.7) patients without viral hepatitis. This risk of HCC was even greater if both obesity and diabetes were present comorbid conditions (OR: 11.8, 95% CI: 2.7-51.9)[40]. A Danish study further confirmed these results, finding a twofold increase in liver cancer incidence in obese subjects compared to non-obese subjects[41].

A meta-analysis of 11 cohort studies that evaluated the association between being overweight or obese and liver

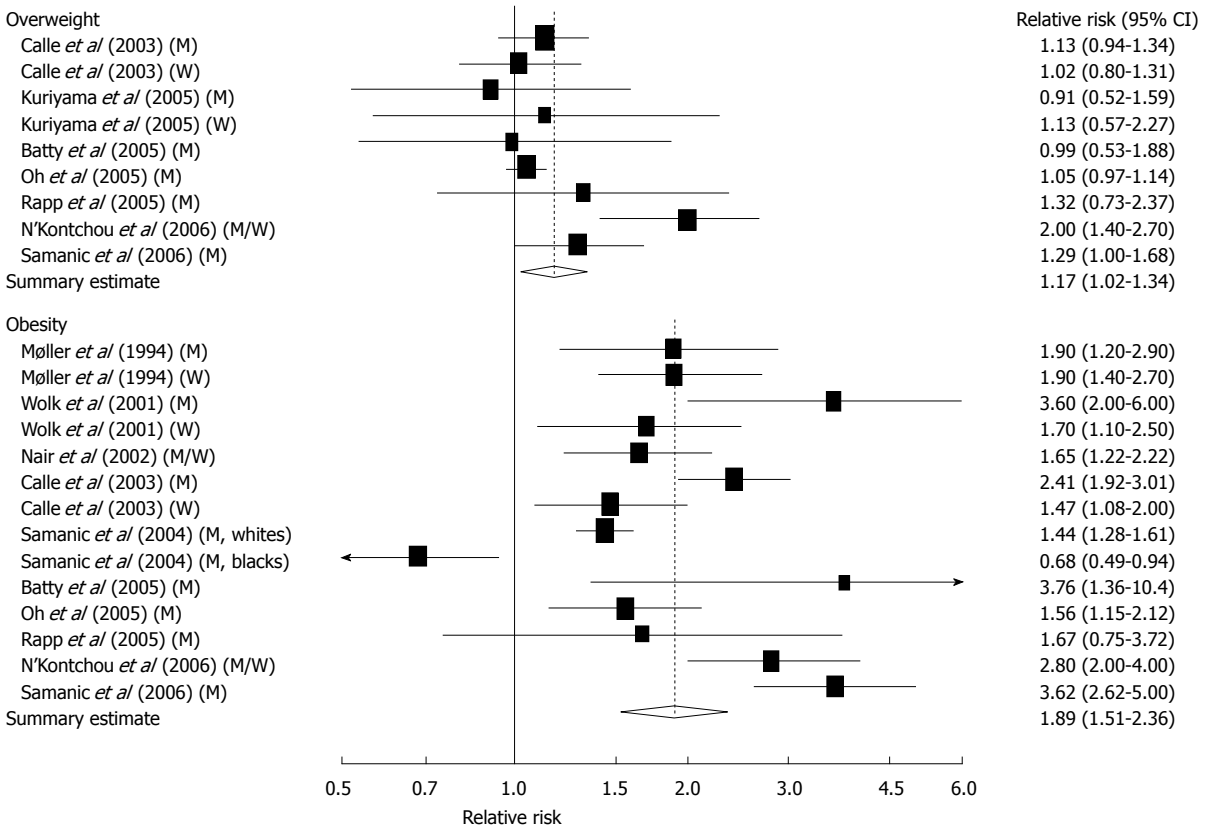

Figure 4 Relative risks of liver cancer associated with overweight and obesity. Relative risk estimates are for overweight and obese persons compared with normal weight persons. Tests for heterogeneity: overweight, Q = 16.83, P = 0.03, I^2 = 52.5%; obesity, Q = 88.03, P < 0.001, I^2 = 86.4%. M: Men; W: Women[42]. Reprinted by permission from Macmillan Publishers Ltd: [Br J Cancer]. Larsson SC, Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. *Br J Cancer* 2007; 97: 1005-1008, copyright (2007)[42].

cancer was published in 2007, and clarified the risk of development of HCC (Table 1)[42]. Of the included studies, seven examined a total of 5037 overweight patients and 10 examined 6042 obese patients[42]. Patients who were overweight had a 17% increase in risk of developing HCC, whereas obese patients had an 89% increase in risk (Figure 4)[42]. Based on the prevalence of HCC, it was estimated that 28% of HCC cases in men and 27% in women were due to being overweight or obese[42].

In addition to an increased risk of developing HCC, overweight or obese patients appear to be at increased risk for HCC-related mortality. In a population-based study of cancer mortality and BMI, men with a BMI of 30-34.9 were found to have a twofold increase in the risk of death from HCC, with a 4.5-fold increase noted in men with BMI > 35[38].

Lastly, *via* the pathway of the metabolic syndrome with resultant NASH cirrhosis, obese patients have been found to be at an increased risk for HCC occurrence. Many lines of evidence point to the role of cirrhosis as a mediator in these patients. Firstly, patients presenting with cryptogenic cirrhosis were found to have a significantly higher prevalence of obesity than patients with cirrhosis from non-alcoholic hepatitis C or autoimmune liver disease, but a similar prevalence of obesity when compared to patients with documented NASH[43]. These data are supported by a case-control study in which 49 patients with cryptogenic cirrhosis were compared to 98 matched controls with an established cause of cirrhosis. In that study, obesity was significantly more prevalent in the cryptogenic cirrhosis patients[44]. Additionally, a retrospective analysis of 19 271 American patients who had undergone liver transplantation found that there were 653 cases of HCC, and those with a diagnosis of cryptogenic cirrhosis had an 11-fold increase in the risk of having HCC[45]. Therefore, being overweight and obesity, secondary to cryptogenic cirrhosis, or more likely undiagnosed NASH cirrhosis, can increase the risk of developing HCC. Clearly, these data suggest that screening is important for diagnosis of asymptomatic HCC and highlight the need for surveillance in this population.

DIABETES

Diabetes has been found to increase the risk of developing chronic liver disease and HCC[46]. Studies that have compared patients with cryptogenic cirrhosis to patients with a known etiology of their cirrhosis have shown a significantly higher prevalence of diabetes among the latter group[43,44]. Again, as noted with the overweight and obese, a similar prevalence of diabetes has been observed among patients with cryptogenic and NASH cirrhosis[43].

In addition to increasing the prevalence of chronic liver disease, diabetes has also been shown to be an independent risk factor for the development of HCC. In a recent systematic review of 13 case control studies, 11

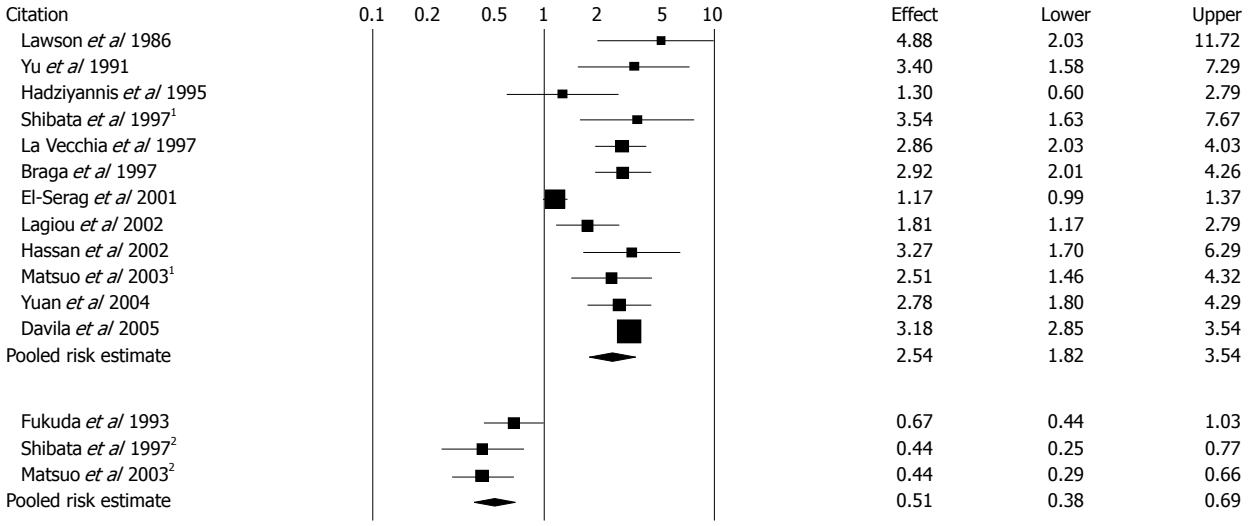

Figure 5 Unadjusted ORs and 95% CIs for the association between diabetes and hepatocellular carcinoma in 13 case-control studies. [1]Population-based control groups; [2]Hospital-based control groups. Black diamonds indicate weighted average of all studies; Black boxes indicate point estimates for ORs[47] Reprinted with El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; 4: 369-380, Copyright (2006), with permission from Elsevier[47].

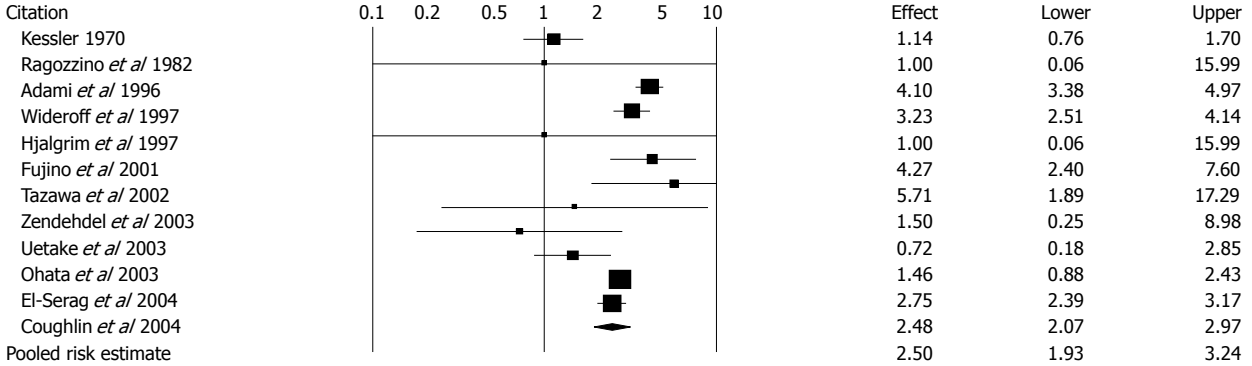

Figure 6 Unadjusted risk ratios and 95% CIs for the association between diabetes and hepatocellular carcinoma in 12 cohort studies. Black diamond indicates weighted average of all studies; Black boxes indicate the point estimates for risk ratios[47]. Reprinted from El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; 4: 369-380, Copyright (2006), with permission from Elsevier[47].

supported an association between diabetes and the development of HCC[47]. Among the 13 case-control studies, subjects with diabetes were found to have a twofold increase in the risk of HCC; an association that was further strengthened by excluding studies with significant heterogeneity (Figure 5)[47]. This association was also appreciated amongst 12 cohort studies evaluated (Figure 6). The presence of diabetes remained an independent risk factor for HCC after adjustment for alcohol use or viral hepatitis in the studies that evaluated these factors[47]. However, as dictated by the limitations of the studies available in the literature, further well-defined studies are required to account for dietary factors and obesity.

DIET

Several studies have examined whether alterations in diet have an effect on the risk of HCC. A trial from Italy has examined a broad range of dietary habits among 185 patients with HCC and 412 patients without cancer[48,49]. Those with HCC were more likely to consume a large amount of calories, were five times more likely to be former drinkers, and were 30 times more likely to be infected with either HCV or hepatitis B virus (HBV). Among dietary compounds, consumption of iron and thiamine were associated with a significant threefold and twofold increase in risk of HCC, respectively. Conversely, β-carotene and linoleic acid consumption was associated with a reduced risk of HCC[48]. An association between intake of iron was also evaluated according to the presence or absence of viral hepatitis[48]. When compared to appropriate controls, consumption of iron among patients without viral hepatitis was associated with a significantly increased risk of HCC[48]. This increase in risk was not conferred to those with HCV or HBV. In a similar study, those subjects with consumption in the highest quartile for yogurt and milk, white meat and eggs had a significantly lower likelihood of developing HCC[50]. This effect was observed in patients with and without viral hepatitis[50].

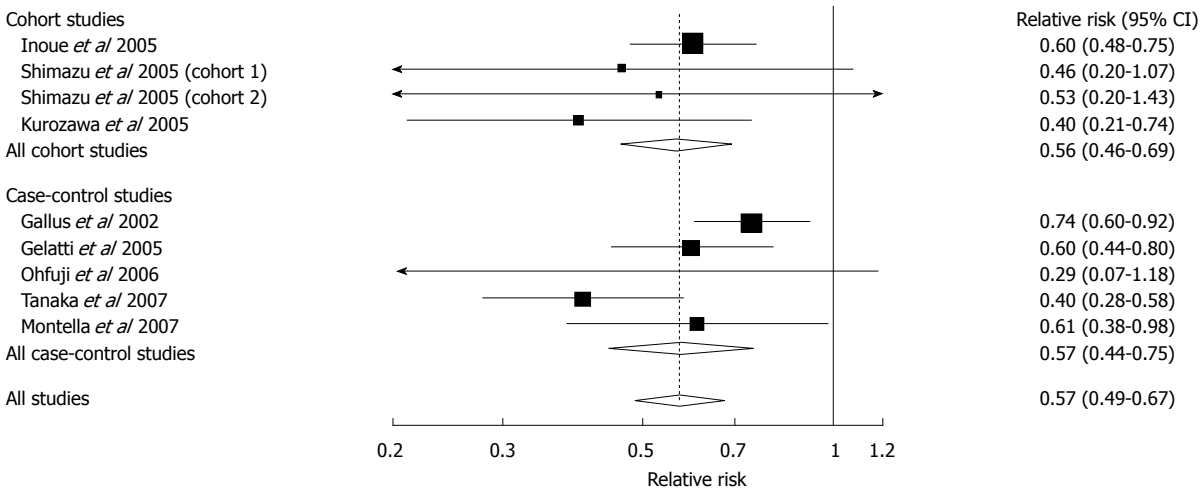

Figure 7 Relative risks of liver cancer associated with coffee consumption (per 2 cups/d increment). Squares represent study-specific relative risk estimates (size of the square reflects the study-specific statistical weight, that is, the inverse of the variance); horizontal lines represent 95% CIs; diamonds represent summary relative risk estimates with corresponding 95% CIs. Tests for heterogeneity: all studies, Q = 11.56, P = 0.17, I^2 = 30.8%; cohort studies, Q = 1.74, P = 0.63, I^2 = 0%; case-control studies, Q = 9.28, P = 0.05, I^2 = 36.9%. Reprinted from Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterology* 2007; 132: 1740-1745, Copyright (2007), with permission from Elsevier[66].

Other studies from Japan and Europe have found those who consume a large amount of green vegetables have a significantly lower likelihood of developing HCC[51-53]. One study has shown that eating green vegetables daily, as compared with consumption fewer times per week, had a protective effect against the development of HCC (OR: 0.75, 95% CI: 0.60-0.95)[51]. On the contrary, a Greek study has found no association between vegetable intake and reduction in the risk of developing HCC[54].

In summary, there is evidence to suggest that consumption of yogurt and milk as well as vitamin supplements offers a protective effect against HCC. The enthusiasm for these findings however should be tempered by the fact that the majority of these studies were retrospective in nature.

COFFEE

In addition to its reported association with reductions in bladder cancer and colorectal cancer, coffee consumption has also been extensively studied and appears to have a potentially favorable effect on the prevention of liver diseases, including HCC[55,56]. There are several hypotheses that could explain why consuming coffee attenuates the risk of developing HCC. One hypothesis argues that coffee intake lowers serum levels of γ-glutamyl transferase (GGT), which is associated with a lower incidence of HCC[56-59]. Coffee consumption has also been linked to a lower incidence of cirrhosis, which is a major risk factor for the development of HCC[56].

An analysis of two large prospective studies of > 70 000 participants in Japan has shown that those who drank one or more cups coffee daily had a significantly lower risk of developing HCC[60]. A case-control study of 2746 people has found that those who drank three or more cups of coffee were 40% less likely to develop HCC[56]. Similar results have also been found in an array of studies conducted in Europe and Japan[60-65].

Additionally, two meta-analyses that have examined the association between coffee drinking and the risk of developing HCC have recently been published. The first was inclusive of four cohort studies and five case-control studies[66]. In the pooled analysis, a 43% lower risk of developing HCC was found for those who drank more than two cups of coffee per day (RR: 0.57, 95% CI: 0.49-0.67) (Figure 7)[66]. The second meta-analysis examined four cohort studies from Japan and six from Japan and Southern Europe[67]. There were differing definitions of low and high coffee consumption, however, the results of the studies were consistent. In summary, those who drank any coffee compared to non-drinkers had a significantly lower risk of HCC (RR: 0.59, 95% CI: 0.49-0.72). The greater the coffee consumption, the greater the attenuation in HCC risk. Low coffee consumption was associated with a 30% reduction in risk and high consumption with a 55% reduction in HCC risk (Figure 8)[67].

Although these results are impressive and consistent, one must consider that the findings of an inverse relationship between coffee consumption and the risk of HCC might be influenced by bias. Coffee metabolism is impaired in cirrhotic livers as compared to the normal liver. This altered metabolism generates an increase in the untoward side effects of the beverage. Therefore, the presence of liver disease might lead affected patients to consume less coffee. This could result in a falsely negative association. Therefore, the potential bias of this association in the liver disease patient cannot be discounted.

ALCOHOL

The mechanism by which alcohol consumption increases the risk of HCC is primarily through the development of cirrhosis. It has been suggested that heavy alcohol consumption of > 80 g/d ethanol for at least 5 years increases the risk of HCC by nearly fivefold[68]. The risk appears to be proportional to the amount of alcohol consumed.

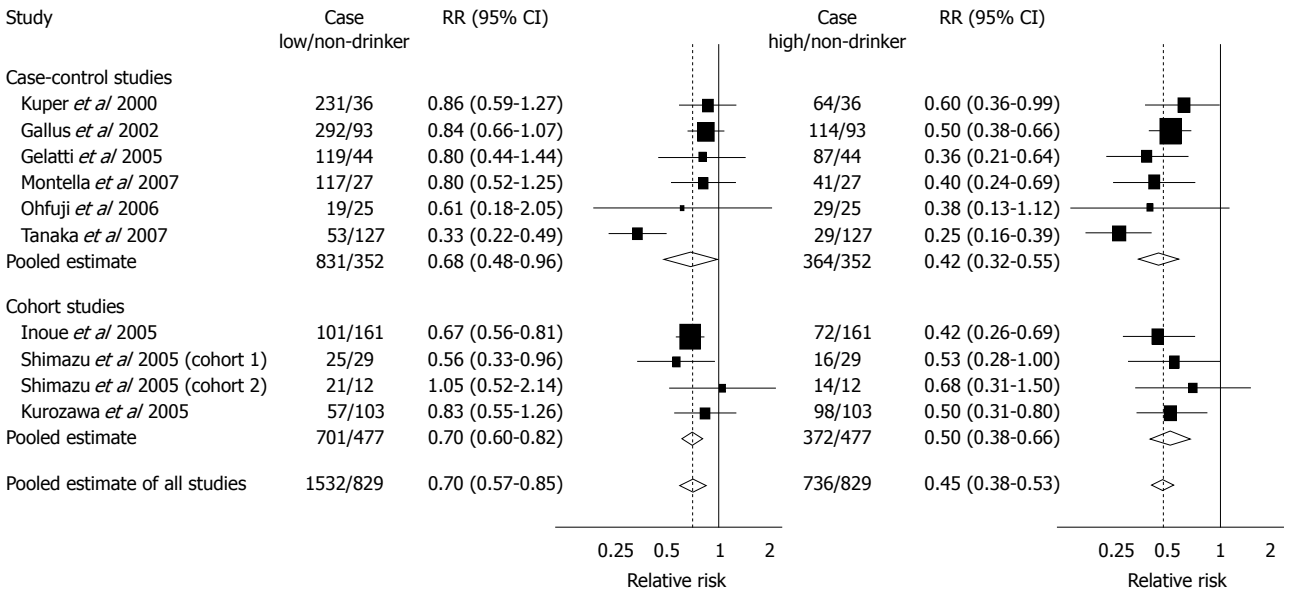

Figure 8 Summary RRs of hepatocellular carcinoma for low or moderate and high coffee drinkers *vs* non-drinkers from case-control and cohort studies. Low or moderate consumption was defined as < 3 cups per day for Gallus *et al*, Gelatti *et al*, Inoue *et al* and Montella *et al* and as < 1 cup per day for Ohfuji *et al*, Tanaka *et al*, Kurozawa *et al*, and Shimazu *et al*; High consumption was defined as ⩾ 3 cups per day for Gallus *et al*, Gelatti *et al*, Inoue *et al*, and Montella *et al* and as ⩾ 1 cup per day for Ohfuji *et al*, Tanaka *et al*, Kurozawa *et al*, and Shimazu *et al*. Bravi F, Bosetti C, Tavani A, Bagnardi V, Gallus S, Negri E, Franceschi S, La Vecchia C. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology* 2007; 46: 430-435[67]. Copyright 2007, John Wiley & Sons, Inc. Reproduced with permission of John Wiley & Sons, Inc.

As characterized by a meta-analysis, relative risks of 1.19 (95% CI: 1.12-1.27), 1.40 (95% CI: 1.25-1.56), and 1.81 (95% CI: 1.50-2.19) were associated with the consumption of 25, 50 and 100 g/d alcohol, respectively[69].

In addition to a daily dose response, persistent alcohol consumption appears to have a long-term effect on the risk of HCC occurrence. A prospective case-control study from Japan has observed that heavy alcohol drinkers, defined as > 600 L of alcohol during a lifetime, had a fivefold increase in the risk of HCC in comparison to non-drinkers or those who consumed < 600 L of alcohol (OR: 5.19, 95% CI: 2.53-10.64)[70]. However, the risk of HCC among those who consume low or moderate levels of alcohol remains unknown[1].

An association between genetic polymorphisms of the enzymes participating in the metabolic pathway of ethanol and the increased risk of HCC in heavy alcohol drinkers has been also proposed as a mechanism by which HCC develops. The frequency of aldehyde dehydrogenase 2 (*ALDH2*) genotype polymorphisms is significantly associated with increased risk of HCC in heavy alcohol drinkers (OR: 2.53, 95% CI: 1.63-58.60)[70]. A study from Italy has observed that, among subjects who consumed > 100 g/d of ethanol and were bearers of the gluthatione S-transferase M1 (GSTM1) null genotype had twice the risk of HCC compared with bearers of the GSTM1 non-null genotype (OR: 8.5, 95% CI: 3.9-18.6 *vs* OR: 4.5, 95% CI: 2.0-10.0)[71].

SMOKING

Several studies have evaluated the association between smoking and development of primary liver cancer. A prospective cohort study including 4050 men aged ⩾ 40 years who were followed-up for an average length of 9 years observed that those who smoked had a threefold increased risk of primary liver cancer when compared to never smokers (RR: 3.3, 95% CI: 1.2-9.5)[72]. Additionally, a study from Korea has found a 50% increase in the risk of primary liver cancer for current male smokers compared to never smokers[73]. In contrast however, a recent population-based case-control study from the United States did not observe a significantly increased risk of primary liver cancer among current male smokers[74]. Male ex-smokers, however, had a significant increase in risk of primary liver cancer, which suggests that there is perhaps a dose or duration response underlying this association[72-74]. Such responses were further explored in the Korean Cancer Prevention study that included 1 283 112 subjects[75]. Although the amount of smoking did not alter the risk of HCC, the duration of smoking significantly increased the risk of HCC for subjects who had smoked for > 20 years when compared to those who had smoked for < 10 years[75].

The association between tobacco and liver cancer and its reliance on host factors such as genetics, sex, and an underlying history of viral hepatitis has also been explored. With respect to the role of genetics, a small study from Japan has evaluated 78 patients with HCC and genetic polymorphisms of tobacco and alcohol-related metabolizing enzymes and 138 hospital controls without cancer. They have demonstrated that cigarette smokers did not have a significantly increased risk of HCC when compared with non-smokers[70]. To analyze the effect of sex, a prospective cohort study that included 83 885 patients followed up for 8 years observed a positive association between smoking and HCC in women who smoked > 10 cigarettes per day (RR: 4.2, 95% CI: 1.3-13.8)[76]. However, no significant increase in the risk of HCC was

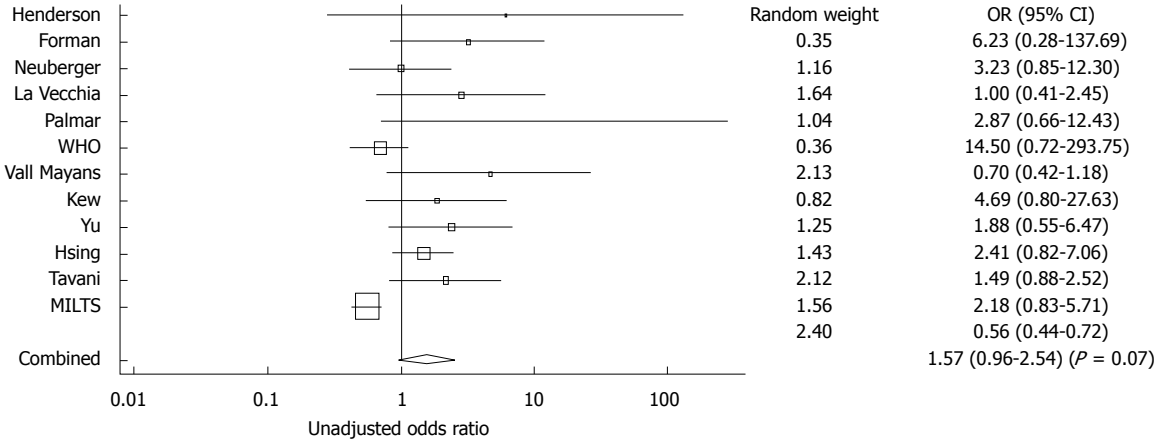

Figure 9 Forest plot showing adjusted OR and 95% CI for the association between oral contraceptive and hepatocellular carcinoma for the eight studies that included adjusted ORs. The diamond symbol indicates the weighted random pooled OR of all studies included in the analysis. Reprinted from Maheshwari S, Sarraj A, Kramer J, El-Serag HB. Oral contraception and the risk of hepatocellular carcinoma. *J Hepatol* 2007; 47: 506-513, Copyright (2007), with permission from Elsevier[95].

demonstrated among male smokers[76]. Additionally, to determine the effect of viral hepatitis on the association between HCC and tobacco, a prospective study of 12 008 men observed that smoking significantly increased the risk of HCC only in anti-HCV-positive patients but not in those who were anti-HCV-negative when compared to anti-HCV-negative nonsmoking individuals[77].

In addition to an increase in the risk of developing HCC, it is also suggested in the literature that smoking increases the risk of death in HCC. In the Korean Cancer Prevention cohort study, men who were current smokers had an increased risk of death from HCC[75]. Women who were current smokers did not have the same elevation in risk of HCC-related death as that observed in men[75]. These findings were further replicated in the Japan Collaborative Cohort (JACC) Study that analyzed 65 528 subjects aged 40-79 years[78]. In this cohort, an increased risk of death due to HCC was shown among current and ex-smokers[78]. Further analyses from the JACC cohort demonstrated that cigarette smoking significantly increased the risk of death from HCC in individuals positive for anti-HCV antibody[79].

ORAL CONTRACEPTIVES

Prior to the widespread use of oral contraceptives (OCs), benign liver tumors in young women were rarely observed. In the current case report literature, however, therapy with oral contraceptives appears to be associated with the development of benign liver tumors such as hepatic hemangioma, hepatocellular adenoma or focal nodular hyperplasia[80,81]. Although not well researched, it has been proposed that OCs might also be associated with malignant liver tumors including HCC[82,83]. Rarely, malignant transformation can occur within the context of hepatic adenomas. It is unclear, however, whether the use of OCs influences the likelihood of developing adenoma and that these benign tumors transform.

Within the literature, there have been 14 cases of hepatic adenoma with focal malignant transformation to HCC in women taking OCs[83-93]. The mean age of these patients at the time of diagnosis of malignant transformation was 36 years (range: 23-57 years) and the mean duration of OC use was 11 years (range: 1 mo-20 years)[83-93]. Although difficult to obtain from the literature, the frequency of HCC among hepatic adenomas appears to vary from 5% to 18%[89,92-94].

To evaluate further the risk of HCC in the setting of OC use, several observational studies have been conducted. A recent meta-analysis of 12 case-control studies, including 739 cases and 5223 controls, which evaluated the risk of HCC among women using OCs indicated that there was no increase in risk of HCC with short-term use; defined as < 5 years of exposure[95]. An analysis of all studies in the aforementioned meta-analysis yielded a pooled unadjusted OR of 1.57 (95% CI: 0.96-2.54)[95]. An adjusted analysis, which accounted for variables such as age, race and parity, did not yield significant findings (Figure 9)[95]. On the contrary, six studies have observed a significantly increased risk of HCC among women taking OCs for > 5 years; an increase in risk of 2-20-fold[95]. However, given the variable periods of duration used in each of the studies, a pooled estimate of risk could not be generated[95]. Based on these results, further studies are required to evaluate the association between OCs and the risk of HCC and how such risk is modified by duration of OC use. Additionally, it should be noted that an association between new-generation OCs with lower doses of hormones and the risk of HCC has not yet been explored.

BETEL QUID

The chewing of betel quid is woven into the cultural fabric of up to 20% of the world population. Betel quid consists of the nut of the *Areca catechu* palm (areca nut), betel leaf or fruit from *Piper betle* and red slaked paste[96]. These ingredients have been shown to have genotoxic, mutagenic and tumorigenic properties[97-102]. A case-con-

trol study from Taiwan has shown that betel quid chewing was an independent risk factor for liver cirrhosis (OR: 3.56, 95% CI: 1.41-8.96)[103].

Recently, two prospective case-control studies from Asia also have observed a significant association between betel quid chewing and the incidence of HCC. One such study included 263 pairs of age- and sex-matched patients with HCC and healthy controls and observed that betel quid chewing was an independent risk factor for HCC, with a threefold risk noted (OR: 3.49, 95% CI: 1.74-6.96). The aggregate risk increased with increasing duration and/or quantity of consumption[96]. These data were further supported by a study from Taiwan, including 420 age- and sex-matched patients with HCC and liver cirrhosis, liver cirrhosis only and healthy controls. In this study, a nearly sixfold and nearly twofold increased risk of HCC was observed in patients with HCC compared with healthy controls and patients with liver cirrhosis, respectively[104]. Additionally, they found an additive interaction between betel quid chewing and chronic HBV and/or HCV infection.

AFLATOXIN

Aflatoxin B1 (AFB1) is the major metabolite of the molds *Aspergillus fumigatus* and *Aspergillus parasiticus*. These molds grow on a variety of food products that are stored in warm and damp conditions or are cultivated in countries with hot and humid climates[1,105]. AFB1 induces a single nucleotide substitution in codon 249 in the *p53* tumor suppressor gene, which results in the change of the amino acid arginine to serine[106,107]. This mutation is present in up to 50% of patients with HCC who are indigenous to geographic regions with high exposure to AFB1[108-111]. On the other hand, this mutation is absent in patients with HCC from regions with low exposure to AFB1[112,113]. Moreover, it has been recently demonstrated that AFB1-albumin adducts in patients with HCC correlate significantly with the presence of plasma DNA hypermethylation and mutations in the *p16* and *p53* tumor suppressor genes[114].

Several studies have evaluated an association between the risk of HCC and exposure to AFB1. A prospective case-control study from China which included 18 244 middle-aged men showed that individuals with the presence of urinary aflatoxin biomarkers had a significantly increased risk of HCC after adjusting for HBV surface antigen seropositivity and cigarette smoking[115]. These data were further supported by a community-based cohort study from Taiwan which found that elevated AFB1 exposure measured by detectable AFB1-albumin adducts was an independent risk factor for HCC after adjustment for important confounders (OR: 5.5, 95% CI: 1.2-24.5)[116].

It should be stressed that areas with high exposure to AFB1 are also characterized by a high prevalence of HBV infection. AFB1 is independent of the risk conferred by HBV, however concomitant exposure to both HBV and AFB1 markedly increases the risk of HCC. When compared to those without HBV infection and absence of urinary AFB1 markers, the risk of HCC was 60 times higher in patients with HBV infection and a concomitant elevation of urinary AFB1 markers (RR: 59.4, 95% CI: 16.6-212.0)[115]. Patients with HBV infection and normal urinary AFB1 markers had sevenfold increase in risk of HCC when compared to appropriate controls[115].

CONCLUSION

Multiple non-viral factors have been implicated in the development of HCC. Hemochromatosis and iron overload syndromes have consistently been shown to dramatically increase the rate of HCC. Additionally, factors such as obesity and diabetes, which operate *via* NASH cirrhosis or perhaps independently, have also been demonstrated to increase the risk of HCC. This phenomenon has closely mirrored the epidemic of obesity over the last 15-25 years.

With respect to other exposures, although alcohol and tobacco clearly increase the risk of HCC development and mortality, other exposures such as coffee and high levels of vegetable consumption may be protective against this condition. Further studies are urgently needed to determine the pathogenesis that underlies the occurrence of HCC in the setting of these exposures, as well as the way in which such risk is modified by environmental and host characteristics such as genetics.

Clarification of relevant non-viral causes of HCC will help to focus clinicians on those risk factors that are modifiable. With more information, future surveillance efforts will be more appropriately targeted toward populations at greatest risk. This multilevel preventative approach will hopefully lead to a reduction in incidence of non-viral HCC, and a decrease in the patient morbidity and mortality as well as the societal economic burden associated with HCC.

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