REVIEW

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Risk factors for hepatocellular carcinoma in patients with non-alcoholic fatty liver disease

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ABSTRACT – Non-alcoholic fatty liver disease is growing in worldwide prevalence and thus, is expected to have a higher number of NAFLD-related hepatocellular carcinoma (HCC) in the following years. This review describes the risk factors associated with HCC in NAFLD-patients. The presence of liver cirrhosis is the preponderant one. Male gender, PNPLA3 variants, diabetes, and obesity also appear to predispose to the development of HCC, even in non-cirrhotic subjects. Thus far, intensive lifestyle modifications, including glycemic control, and obesity treatment, are effective therapies for NAFLD/ non-alcoholic steatohepatitis and, therefore, probably, also for HCC. Some drugs that aimed at decreasing inflammatory activity and fibrosis, as well as obesity, were studied. Other data have suggested the possibility of HCC chemoprevention. So far, however, there is no definitive evidence for the routine utilization of these drugs. We hope, in the future, to be able to profile patients at higher risk of NAFLD-HCC and outline strategies for early diagnosis and prevention.

Keywords – Hepatocellular carcinoma; non-alcoholic steatohepatitis; non-alcoholic fatty liver disease; NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the commonest chronic liver disease worldwide, with an estimated prevalence of 25% worldwide, and can progress to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC). The aspects that lead to NAFLD progression are only partially understood. It is a significant limitation since almost 50% of HCC cases occur in patients without cirrhosis[1].

Furthermore, the non-alcoholic fatty liver disease and non-alcoholic steatohepatitis are associated with about 30–40% of HCC worldwide[2-5]. HCC incidence and prevalence rates in cirrhotic and non-cirrhotic NAFLD/NASH vary among studies; data have shown that 35.5% of NAFLD-related HCC occur in patients with bridging fibrosis or cirrhosis[2-5]. The HCC also appears to be associated with risk factors for metabolic syndrome, despite the progression of liver fibrosis. Of note, obesity and type 2 diabetes mellitus (T2DM) are the main risk factors for NAFLD/NASH[6].

HCC has an annual incidence in cohorts that included NASH cirrhotic patients in the US and Europe that varied from 0.7 to 2.6%. Studies from Indian, Brazilian, and Japanese subjects have shown incidences of 0.5% per year, 4% in 5 years, and 11.3% in 5 years, respectively. In non-cirrhotic patients with NAFLD/NASH, the incidence ranged from 0.22 to 1.32 /1000/year in European countries and from 0.21 to 10.6/1000/year in the USA[1]. A systematic review and meta-analysis of 19 studies showed a prevalence of HCC in NASH non-cirrhotic patients at around 38%, but among patients with non-cirrhotic NAFLD, the observed prevalence was 14%[1]. According to another meta-analysis, in patients with simple steatosis, HCC had an incidence of 0.44 per 1000 person-years, whereas, in NASH patients, this rate reached 5.29 per 1000 person-years[1].

There are several risks associated with HCC related to NASH. They include advanced liver fibrosis, older age, male gender, and metabolic syndrome. Other risk factors that may have an association with NASH-related HCC are genetic factors and dietary patterns. Therefore, systematic HCC surveillance, as performed for patients with chronic liver disease due to other etiologies, might be required for NAFLD-patients. Of note, a simple, rapid, and specific detection of NASH-related HCC require the development of diagnostic markers[7].

Hence, the challenge is to detect risk factors associated with the development of HCC in NAFLD patients and cost-effective strategies for screening and diagnosis.

Hyperinsulinemia

There is hyperactivation of insulin-dependent signaling pathways in individuals with HCC tumors; it appears to be related to insulin signaling. Because of the signaling component overexpression and the loss of negative regulators, this pathway seems to be frequently altered and upregulated in many cases. Since hyperinsulinemia acts in the cancer cells metabolism, proliferation and survival, it can directly affect the HCC development[8-10].

Hyperinsulinemia in NAFLD-patients is related to decreased liver clearance of insulin. In this context, the mechanisms of hepatic aggression include systemic inflammation with activation of pro-inflammatory pathways of cytokine release such as TNF-

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a and IL-6, imbalance in adipokine secretion, lipotoxicity, and alterations in the intestinal microbiota, mitochondrial injuries, and oxidative stress[9]. These mechanisms contribute to damage insulin signaling at both receptor and post-receptor levels by starting a variety of serine/threonine kinases (including c-Jun amino-terminal kinase, jB kinase-b inhibitor, conventional and novel protein kinases C, mTORC1/ S6 kinases) and MAPKs that promote inhibitory phosphorylation of RI and its key substrates (IRS-1; IRS-2). Initiation of transmembrane and cytosolic phosphoprotein phosphatases that are negative insulin regulators, e.g., PP2A, PTP1B, and SHP2, have also been described in insulin-resistant states. Proinflammatory cytokines contribute to cellular insulin resistance by inducing the expression of proteins SOCS-1 and SOCS-3 that avoid IRS-1 and IRS-2 tyrosine phosphorylation and promote IRS degradation[9].

NAFLD has a clear association with insulin resistance and a higher insulin-like growth factor (IGF-1). Activation of the liver gene and the IGF receptor at its 1R binding to IGF3 and the AKT and kin receptors occurs. In addition, there is the activation of a protein that stimulates the activation of Wnt/β-catenin for fibrosis by mitosis/AK receptor, activating MAPK. and risk of carcinogenesis[9].

The increase in lipid stress levels and reactive oxygen species (ROS) of the pathways may infer excessive resistance and its consequences. Increased ROS and apoptotic pathways lead to necroinflammatory activity and hepatic fibrosis. Mitochondrial activity and ROS production might lead to NASH and HCC in NAFLD patients.

Obesity

In the context of metabolic syndrome, the obesity is a relevant risk factor for NAFLD and its consequences, such as HCC. About 90% of obese individuals may have NAFLD, and around 30% have NASH[11]. Worldwide, an increase in the incidence of liver cancer is observed in parallel with the prevalence of obesity[12].

There are numerous explanations why obesity is a risk factor for HCC. Data have shown that obesity is related to insulin resistance and an increased insulin-like growth factor that is a cell growth-triggered by mitogen.

A meta-analysis found that being overweight was an independent risk factor to liver cancer. Of the 11 cohort manuscripts considered, seven included overweight people (n=5037) and ten included obese people (n=6042)[12]. Compared with normal-weight subjects, the HCC relative risks were 1.17 (95%CI: 1.02–1.34) for those with overweight and 1.89 (95%CI: 1.51–2.36) for those who were obese[12].

Some studies have applied BMI as a criterion for obesity diagnosis, even when considering cirrhosis and ignoring the presence of ascites. In these studies, possible confounding variables could be advanced chronic liver disease and obesity and, therefore, should be controlled since biases could occur after analysis.

Another study evaluated 19,271 patients; overall, the incidence of hepatocellular carcinoma was 3.4% (n=659); obesity was an independent predictor for liver cancer in patients with alcoholic cirrhosis (OR 3.2; 95%CI, 1.5–6.6; *P*-0.002) and cryptogenic cirrhosis (OR, 11.1; 95%CI, 1.5–87.4; *P*-0.02)[13]. Data have shown that some subjects with cryptogenic cirrhosis had NAFLD etiology[14].

An analysis performed on 23,820 people followed for 14 years observed that obesity was independently linked with an enlarged HCC-risk (RR 4.13; 95%CI, 1.38–12.4) in hepatitis C positive patients but not in those with hepatitis B. People without hepatitis B and C had a two-fold increased chance of liver cancer after adjusting for other variables (RR, 2.36; 95%CI, 0.91–6.17)[15]. Diabetes was also connected to HCC regardless of the presence or absence of viral hepatitis[15].

Obesity is a significant health problem. It is associated with other cancers such as breast, bladder, colon, renal cell carcinoma, and esophageal adenocarcinoma[13]. Obesity is also related to greater levels of estrogens, a well-recognized risk factor for hepatocellular adenoma[13].

Diabetes mellitus type-2

Diabetes has a harmful role in patients with liver diseases and it is associated with cirrhosis progression in NASH-subjects and a greater risk of liver cancer in NASH and NASH-cirrhosis people[10]. In observational studies, Diabetes type 2 presence had an increased risk of developing HCC; the greater risk ranged from two to four times. Some studies also adjusted HCC and Diabetes association for possible confounding factors, as alcohol intake and viral hepatitis. Obesity and diabetes are conditions included in the Metabolic Syndrome, and thus, their independent contribution is hard to establish since both are strongly associated, including from a pathogenic viewpoint[16].

A retrospective study included 6,508 Japanese subjects with NAFLD detected by ultrasonography and with a median follow-up of 5.6 years. In all, 16 (0.25%) new cases with HCC were diagnosed during the study and the multivariate analysis identified diabetes (HR: 3.21; 95%CI: 1.09–9.50; *P*=0.035), serum AST level ≥40 IU/L (HR: 8.20; 95% (95%CI): 2.56–26.26; *P*<0.001), platelet count $<150 \times 10^{(3)}$/μl (HR: 7.19; 95%CI: 2.26–23.26; *P*=0.001) and age ≥60 years (HR: 4.27; 95%CI: 1.30–14.01; *P*=0.017) as independent risk factors for HCC[17].

One study evaluated patients with NASH-cirrhosis at Mayo Clinic Rochester as well as adult NASH liver transplanted registrants between 2004 and 2017 using the United Network for Organ Sharing (UNOS)/Organ Procurement and Transplantation Registry for external validation. Among 354 Mayo Clinic patients with NASH cirrhosis, 253 (71%) had diabetes, and 145 (41%) were male. During a median follow-up of 47 months, 30 subjects presented HCC. Diabetes was associated with an increased risk of developing HCC in univariate (HR=1.4; 95%CI =1.1–1.8; *P*<0.01) and multivariable (HR=1.3; 95%CI =1.0–1.7; *P*=0.03) analysis[18]. Additionally, an international multicenter cohort including 458 patients with NASH biopsy-proven, stage 3-fibrosis and cirrhosis, with mean follow-up of 5.5 years, found Diabetes to be a strong predictor of HCC (HR 4.72)[19].

An Italian multicenter case-control study aimed to separately explore the association between risk of liver cancer and features of metabolic syndrome. 185 HCC-cases were compared with 404 controls and the OR to HCC in Diabetes group was 4.33 (95%CI 1.89–9·86), and there was no association between arterial hypertension or hypercholesterolemia and HCC. The OR for overweight and HCC (BMI ≥25 kg/m²) was 1.25 (95%CI 0.72–2.18), and for obesity (BMI ≥30 kg/m²) was 1.97 (95%CI 1.03–3.79)[20].

Cirrhotic versus non-cirrhotic patients

Data on risk factors for HCC in non-cirrhotic patients are infrequent. Advanced liver fibrosis is associated with most liver-related complications, including HCC and shorter survival in NASH patients, obesity, insulin resistance, and pro-inflammatory NAFLD/NASH reactions can lead to carcinogenesis even in the absence of cirrhosis[6,21-23].

Analyzes of HCC-NASH in non-cancerous tissue showed advanced fibrosis in about 80% of patients who progressed to HCC, which is the preponderant risk factor (odds ratio, OR, 4.23)[24]. On the other hand, another study identified HCC in only 46% of NAFLD patients who had cirrhosis in non-cancerous tissue[24,25]. Thus, there is a significant proportion of NAFLD-related HCC patients without advanced fibrosis; despite frequent findings of fatty infiltration and inflammation in the liver tissue. A prospective study followed 253 patients with NASH-cirrhosis for 47 months. Of these, 30 (11.86%) patients developed HCC. DM2, advanced age, and hypoalbuminemia were related with a greater chance of HCC[1].

Among patients with NASH, male gender and older adults are associated with a rising in HCC risk[26]. Men with metabolic syndrome may also be at increased risk of HCC, even in the absence of advanced liver fibrosis (stage F0-F2 for liver fibrosis)[27]. A Japanese study included 87 patients with NASH-HCC and observed that men had HCC in a less advanced stage of fibrosis than women[26]. Other analysis demonstrated that, in the absence of cirrhosis, T2DM, dyslipidemia, and arterial hypertension were independent risk factors for HCC and, possibly, metformin and lipid-lowering medications decreased the risk of developing liver cancer[28]. There is a higher prevalence of T2DM, obesity, arterial hypertension, coronary artery disease, and dyslipidemia in patients with HCC and non-cirrhotic NAFLD when compared to patients with non-cirrhotic HCC of another etiology[29].

However, despite metabolic syndrome being a risk factor for HCC in non-cirrhotic patients, the pathways that lead to carcinogenesis are still not fully elucidated[30]. NAFLD-related HCC may have a worse prognosis because there is no cancer screening in non-cirrhotic, even though the data showed that the 1-year survival rate did not differ between NAFLD-related HCC and other etiologies[26].

Simple steatosis versus non-alcoholic steatohepatitis

Obesity, T2DM, iron overload, and alcohol consumption are risk factors for HCC in the NAFLD[31]. Although the HCC carcinogenesis in NAFLD/NASH is still not fully understood, data have shown that the obesity-related pro-inflammatory state may be associated with the release of substances such as tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and leptin, relevant factors for carcinogenesis[6]. Additionally, there is evidence of an association between microbiota disbalance, obesity, NAFLD, and HCC.

Data suggested that T2DM and obesity can double the HCC-risk; the value, however, that each of these variables adds when put together is unknown[30]. T2DM and insulin resistance associated with hyperinsulinemia and IGF-1 levels also appear to contribute to the carcinogenesis of HCC. Patients with diabetes have a 2- to 4-fold increased risk of developing HCC, regardless of association with another cause of liver damage, such as viral hepatitis or alcohol use, and possibly is associated with an increased risk of cancer recurrence after curative therapy[30].

Hepatocarcinoma is the leading cause of cancer-related death in obese middle-aged male patients, and obesity can increase HCC-associated mortality. Obesity is a risk factor for other cancers. A meta-analysis performed with 11 cohort studies found an association between overweight, obesity, and HCC with a relative risk of 1.2 and 1.9, respectively[30,32].

Gender

Male gender may be an HCC risk factor, despite cirrhosis. Perhaps, estrogen is a protective factor against HCC progression, but the exact mechanism is not clarified. Data showed that estrogen receptor β expression is downregulated in HCC tissue compared with normal liver tissue; additionally, its expression level presented a significant negative association with disease development and a positive link with the expression of NLRP3 inflammasome components levels[33]. Treatment with 17β-estradiol (E2) significantly inhibited the malignant behavior of HCC cells through E2/ERβ/MAPK pathway-mediated upregulation of the NLRP3 inflammasome[33].

It has already been demonstrated in animal models that E2 could prevent tumor growth through regulation of the polarization of macrophages. The E2 appears to work as a macrophage alternative activation and tumor progression suppressor by keeping estrogen receptors away from interacting with ATP5J, thus inhibiting the JAK1-STAT6 signaling pathway. These findings possibly indicate a new mechanism for suppressing male-predominant HCC[34].

Of note, it may be hard to differentiate clinical aspects related to metabolic syndrome influenced by sex and ethnicity and their association with HCC. Body fat distribution of men and women is different and the ethnic-geographical influence on this variable is difficult to measure, as is how much it can contribute to the progression of HCC[35].

Ancestry / race

The epidemiology of NAFLD is better characterized among Caucasians[36,37]. Some studies assessed the NAFLD ancestry variation and found a higher prevalence among Hispanics and Latin Americans but lower among African Americans[37]. However, the reasons for these variances are unclear; epigenetic, environmental, and metabolic risk factors are not enough to justify this variation[37]. Among subjects with NASH confirmed by histology, compared to no-Latino whites, the Latins were younger, ate more carbohydrate calories, were less engaged in physical activity, and had lower income and lower prevalence of hypertension[35]. Concerning the NASH severity, there wasn't a significant difference between Hispanics and Caucasians with biopsy-proven NASH. However, among Hispanic diabetic patients, there is an increased risk for fibrosis. African Americans have a low prevalence of hepatic steatosis (24%) while Latin Americans have the highest (45%). White individuals have an intermediate prevalence of 33%[38]. The high prevalence of obesity and insulin resistance among Latin Americans can justify the increased prevalence of hepatic steatosis in this ethnic group[38]. Asians/Pacific Islanders and Hispanics have 3-fold and 2-fold higher reates than the rates among non-Hispanic whites, respectively[39,40].

Furthermore, the relationship between obesity and HCC risk may differ according to ancestry, with a greater association among Americans, Japanese, whites, and Latin American men but not in African-descendant men; and may be related to ancestry and gender variances in fat body distribution[35]. A previous study suggested that obesity was positively associated with HCC in whites, but not in African Americans[1,41]. Compared to whites in similar conditions, Latinos and Asians are more likely than African Americans to accumulate fatty cells in the abdominal visceral compartment and the liver[1,41]. Visceral adiposity has been suggested to be more important for predicting HCC risk than total adiposity[1,41]. A large cohort of NAFLD patients showed a lower risk of HCC in cirrhotic African Americans and cirrhotic and non-cirrhotic non-Hispanic whites compared with Hispanics with cirrhosis. Consequently, it is possible that ethnicity, health behavior, and socioeconomic status be correlated with HCC-NASH[42].

In Japan, the NASH-related HCC rate is thought to be about 3 % of overall HCC, a low rate when compared with the United States[7]. Most cases of HCC in Japan were associated with chronic liver disease caused by hepatitis C virus (HCV)[7]. However, HCV-associated HCC is decreasing, while non-B and non-C HCC (NBNC-HCC), which is negative for HCV and hepatitis B virus infection, has increased[7]. The main cause of NBNC-HCC used to be alcoholic liver disease, but the recent data showed an increased rate of NBNC-HCC in patients with NAFLD[7].

Genetic

Genetic markers may influence HCC risk in NAFLD patients. The *PNPLA3I148M* polymorphism was associated with NAFL and NASH. The *PNPLA3* (rs738409) was related to hepatocellular carcinoma in NAFLD subjects and the genotype proportions were significantly diverse between 100 NAFLD HCC (CC=28, GC=43, GG=29) and 275 controls with NAFLD (CC=125, CG=117, GG=33)[43]. Data were adjusted for sex, DM2, BMI, age and cirrhosis, the G allele increased the risk for HCC (OR 2.26); and HCC risk among GG homozygotes was five times higher than CC[43]. These findings indicate that genetic variations may guide the choice of patients who may have benefits from surveillance, based on risk stratification, regardless of the presence of cirrhosis[44].

The *PNPLA3I148M* works as a repressor of lipid droplet lipase activity, opposing for a shared coactivator[45-47]. This directs that dropping *PNPLA3* expression levels can possibly decrease its negative effect on hepatic lipolysis. Based on this hypothesis, a reduction of *PNPLA3* expression levels secondary to genetic variants make the individual less susceptible to the effect of *I148M* on liver fat than those without the expression-reducing variant[45-48]. It is believed that an etiological distinction can be made between *PNPLA3 I148M*-associated NASH and other forms of NASH that are mainly driven by insulin resistance. Other genes associated with NASH, including *HSD17B13, TM6SF2 (rs58542926 c.449C>T), GCKR (rs780094 A>G / rs1260326 C>T), MBOAT7 (rs641738 C>T), HSD17B13 (rs72613567), IRS1 G972R (rs1801278 A >C), IL28B (rs12979860 C>T)* have been associated with progression to fibrosis and HCC, but analyzes are still needed to prove the potential of these polymorphisms as prognostic markers and future targets for therapeutic intervention.

The human superfamily six transmembrane two (*TM6SF2*), rs58542926, is associated with hepatic triglyceride content and its impact on the cardiovascular system, and higher chance of developing NAFLD and HCC[49]. The *TM6SF2* deletion impairs VLDL secretion, promoting liver steatosis, fibrosis, and accelerated development of liver cancer[49]. In HCC models, neonate mice injected with streptozotocin (NASH/STAM) and fed either high-fat or diethylnitrosamine injection plus fibrogenic diet feeding, TM6 LKO mice exhibited increased steatosis, increased tumor burden, and increased tumor area versus TM6 phlox controls. However, TM6 LKO mice injected with diethyl nitrosamine and fed a fibrogenic diet administered with either wild-type TM6 or TM6 AAV8 mutant E167K showed noteworthy tumor reduction, with tumor burden inversely linked with TM6 protein levels[50].

Analysis associated greater expression of *CEP192* in HCC cell lines than in normal liver cell lines. It is believed that *CEP192* gene expression was upregulated in NAFLD patients, acting as a carcinogenic factor[50]. The survival and validation analysis of gene expression in the TCGA database between HCC and normal samples showed that *CDK1, HSP90AA1, MAD2L1, PRKCD, ITGB3BP, CEP192, and RHOB* were significantly and differentially expressed, with a link between the oxidative stress and pathological polyploidization during NAFLD, which resulted from inactivation of the cyclin B1/CDK1 complex[50]. During HCC progression, up-regulation of the chromodomain helicase/ATPase DNA-binding protein 1-like gene (*CHD1L*) decreases CDK1 activity, progressing mitotic output. Other studies showed an association between high *HSP90AA1* expression, *Mad2l1* loss, *PRKCD* suppression, *and RHOB-VEGFA-VEGFR2* angiogenesis pathways significantly connected with the presence and poor prognosis in HCC.

The genetic markers can be a screening tool to identify patients at increased risk of HCC-NAFLD and a possible therapeutic target.

Iron overload

Iron overload has been observed in patients with HCC. The NAFLD-associated hepatic iron overload may contribute to carcinogenesis due to oxidative stress[30]. Moreover, hyperferritinemia may be a factor that increases the risk of progression of liver fibrosis and HCC in NASH; thus, patients with elevated serum ferritin levels might be screened for HCC[31]. Although there is some evidence of iron overload being a risk factor for HCC, we cannot confirm that this fact is a cause or consequence of advanced liver disease.

Smoking

The incidence of advanced liver fibrosis is higher in smokers. Data suggests that smoking may worsen the NAFLD progression, partly through its action on insulin resistance[51]. Cigarette smoking is an expressive risk factor for the development of HCC, and associations have been found between dose and duration of tobacco use and the risk of HCC, which may have a 1.85-fold higher risk of HCC associated with smoking long-term when compared to individuals who never smoked[52]. Moreover, smoking appears to increase 2-fold risk of NAFLD related HCC and fibrosis-advanced evolution[29].

Medication and lyfestyle

The pathways leading to the development of HCC in patients with nonalcoholic fatty liver disease and nonalcoholic steatohepatitis are complex and in part understood. Although progression to cirrhosis precedes the development of HCC in most etiologies of chronic liver disease, liver cancer can occur in the absence of cirrhosis, particularly in NAFLD.

Strategies to reduce weight and body fat have been identified as the most effective NASH therapy. Lifestyle modification with diet and exercise is the most recommended and effective treatment, but the long-term impact needs to be evaluated. Control of factors associated with metabolic syndrome is recommended, with adequate control of T2DM and dyslipidemia, which, indirectly, can impact the outcome of progression to HCC[53]. Smoking cessation and alcohol consumption are also important, as they are risk factors for cancer of other organ. Prospective cohort study with 63,257 Chinese people and 17.7 years of follow-up, 561 participants developed HCC and concluded that healthy lifestyle has a protective role in HCC development, especially for individuals without hepatitis B or C virus infection (HR 0.13; 95% CI 0.06–0.30) In.[54]

Some studies have pointed out some diet pattern benefits (p. ex. Alternative Healthy Eating Index-2010 and Mediterranean diet), such as weight loss, decrease in glycemic indices and lipid profile, improvement in cardiovascular risk, anthropometric variables,

markers of liver injury severity, and a significantly lower risk of HCC[53,55]. In the EPIC cohort, the multivariable-adjusted HR of HCC was 0.55 (95%CI 0.38–0.80) comparing active and inactive individuals and regarding vigorous physical activity, for those reporting more than 2 hours/week compared to those with no vigorous activity, the HR for HCC was 0.50 (95%CI 0.33–0.76)[56].

Coffee drinking has been inversely related to HCC, and chronic liver disease (CLD) risk. Data suggest that increased coffee consumption is associated with a lower risk of liver cancer, with an inverse correlation between coffee consumption and HCC[57-60]. Among 384,818 coffee drinkers and 109,767 non-coffee drinkers, there were 3,600 cases of CLD, 5,439 cases of CLD or steatosis, 184 cases of HCC, and 301 CLD deaths in a 10.7-years follow-up. Coffee drinkers had lower HR adjusted for CLD (HR 0.79, 95%CI 0.72–0.86), CLD or steatosis (HR 0.80, 95%CI 0.75–0.86), death by CLD (HR 0.51, 95%CI 0.39–0.67) and HCC (HR 0.80, 95%CI 0.54–1.19); those analyzes also included decaf, instant, and ground coffee with similar results[58]. Other meta-analysis showed that HCC relative risk (RR) was 0.66 (95%CI 0.55–0.78) for regular coffee, 0.78 (95%CI 0.66–0.91) for low, and 0.50 (95%CI 0.66–0.91) 0.43–0.58) for high coffee consumption, respectively, and the RR for an increment of one cup per day was 0.85 (95% CI 0.81 –0.90)[59]. A stronger association was identified with coffee and chronic liver disease, with RR 0.62 (95%CI 0.47–0.82) for regular consumption, 0.72 (95%CI 0.59–0.88) for low, 0.35 (95%CI 0.22 0.56) for high and 0.74 (95%CI 0.65–0.83) for an increment of one cup per day[59].

It has not been already proven that weight loss reduces HCC associated with NAFLD, but it does improve the inflammatory processes associated with NAFLD/NASH. Patients who had a 10% reduction in body weight by lifestyle modification had NASH resolution in 90% of cases and fibrosis regression in 45% after 52 weeks of follow-up[61].

Among drug therapies, to date, none has a direct action on the HCC in NAFLD; however, considering the histological improvement of the hepatic and metabolic syndrome, there is an indirect potential to affect this risk. The frequency of NASH and risk of HCC among T2DM patients is significant. Consequently, efforts have been directed towards identifying which antidiabetic drugs may be effective for treating NASH, preventing the progression of liver fibrosis, HCC, or mortality, but without increasing the risk of adverse events.

Vitamin E is a first-line agent for NASH without T2DM. Few observational studies have evaluated the effect of protective micronutrients against HCC, including vitamin E, with no conclusive findings. One included 132,837 participants in China, with a median follow-up of 10.9 years for women and 5.5 years for men, 267 participants developed HCC[62]. The most noticeable finding of this study is that oral intake of vitamin E was associated with a reduced risk of developing HCC[62]. A possible explanation for this result was the varied effects of different subtypes of tocopherols in preventing cancer. In most clinical trials, α-tocopherols are the main components of oral vitamin E supplements.

Pioglitazone has an established role in NASH histology in patients with T2DM. However, its side effects, like body weight gain, fluid retention, cancer incidence, and bone fracture are a concern. There may be a negative association between HCC and thiazolidinediones length of use in type 2 diabetic patients[63].

Associated data on the use of glucagon-like peptide 1 (GLP-1) receptor agonists and sodium-glucose cotransporter 2 (SGLT2) inhibitors demonstrate improvement in NASH and NAFLD. Studies carried out with cell culture and in mice showed that liraglutide suppressed liver carcinogenesis[64,65]. The antitumor activity was mediated by nature killer (NK) cells, but not by CD8+ T cells. Furthermore, liraglutide increased NK-mediated cytotoxicity by suppressing the IL-6/STAT3 signaling pathway in HCC cells[65].

In several meta-analyses, metformin use was associated with an approximately 50% reduction in the risk of HCC, regardless of the etiology of liver disease; however, studies are heterogeneous, especially regarding the definition of NASH[66]. A systematic review and meta-analysis of ten studies including 4,298 HCC patients in a total population of 1,459,417 patients found that statin use was associated with a significantly reduced risk of HCC[67]. A post hoc analysis of 22 randomized trials did not show any benefit from statistics regarding the risk of HCC[68]. We should consider that most of these studies did not have the finding of HCC as a primary outcome. A prospective analysis involving 300,504 participants found a significantly lower risk of HCC among self-reported aspirin users compared with non-users (RR 0.59, 95%CI 0.45–0.77), with no dose-response relationship or taking into account the effect of concomitant use of statins[69,70]. Other studies confirm these aspirin HCC-related findings. There are still no data to support chemoprophylaxis with these medications in patients with NASH and the risk of HCC.

Drug treatment options for NAFLD and specifically for NASH-related HCC are limited. If NAFLD or NASH diagnosis is made before the onset of advanced fibrosis, treatment may be directed at preventing its occurrence. While no drugs have been approved to reverse fibrosis in NASH patients, several treatment regimens have shown promise.

NAFLD-related HCC in Asian countries

In Asian countries, the risk factor most associated with HCC is still chronic infection with the B and C viruses. However, this trend has changed in the last decades. The incidence of NAFLD in this region has increased from 25% in 2000 to 34% currently, with Asian countries contributing 48.3% of the global incidence of NAFLD-associated liver complications in 2019[71-73]. In this population, approximately 50% of patients with NAFLD-related HCC do not have cirrhosis at the diagnosis[71]. Furthermore, in patients with NAFLD-related HCC, the mean age of cancer diagnosis is around 70 years, whereas, in patients with HBV-related HCC, the age group is nearly 50 years[6]. A possible explanation for this feature is that NAFLD is a disease with an insidious course and slow progression[71].

Some studies have already demonstrated this change in the epidemiology direction of NAFLD-related HCC in Asian patients. A Japanese survey of 33.379 cirrhotic patients showed that 31.5% of those with NASH had HCC[74,75]. Another study from South Korea showed that between 2001–2005 and 2006–2010 period, the proportion of patients with HCC associated with NAFLD ranged from 3.8% to 12.2%[76].

This increase in the prevalence of NAFLD and NAFLD-related HCC in Asians in recent decades can be explained by some factors. First, changes in eating habits have made obesity an epidemic on this continent[76]. Furthermore, even with a lower BMI, Asian people have mostly a central distribution of body fat that increases their HCC predisposition. For this reason, the BMI cut-off that represents a risk of HCC for these patients is 23 kg/m²[77]. Another factor influencing the NAFLD incidence in this population is the PNPLA3 variant which is more common in East Asian people

than Black and Caucasian people. This fact can explain the high incidence of NAFLD in this population despite the absence of these metabolic diseases[71].

Lean-NAFLD

Lean-NAFLD has unique results in demographic, biochemical and blood tests, and adds significant risk for diabetes, hypertension and MS in lean individuals[78]. Lean and obese-NAFLD people have several metabolic abnormalities but they show variances in genetic predisposition, body composition, gut microbiota, and susceptibility to environmental factors. Recent data suggest that individuals with lean NAFLD, despite the absence of obesity, have similar cardiovascular and cancer-related mortality compared to obese NAFLD and a higher risk of all-cause mortality[79].

Patients with NAFLD are shown as older, with higher BMI, waist circumference, blood pressure, fasting glucose, insulin, blood lipids, liver enzymes, and uric acid than controls. Although patients with lean NAFLD had lower BMI and waist circumstances, they had a significantly higher visceral adiposity index than overweight and obese controls. Lean NAFLD patients had triglycerides, cholesterol, and low-density lipoprotein cholesterol comparable to overweight and obese NAFLD patients. Lean-NAFLD was most strongly associated with diabetes (OR=2.47, 95%CI: 1.14–5.35), hypertension (OR=1.72, 95%CI: 1.00–2.96), and metabolic syndrome (OR=3.19, 95%CI: 1.17–4.05)[78]. Patients with NAFLD were more likely to have central obesity (OR=1.97, 95%CI: 1.38–2.80), especially in the lean groups (OR=2.17, 95%CI: 1.17–4 .05)[78].

In the Japanese population, the incidence of NAFLD, HCC, and overall mortality was 23.5, 7.6, and 5.9 per 1,000 person-years, respectively. Patient data showed a prevalence of lean NAFLD of 20.7% among the NAFLD population, with people with lean NAFLD being older and having a higher all-cause mortality rate (8.3 vs 5.6 per 1,000 person-years for non-lean NAFLD, P=0.02)[80].

Thus, although the presence of NAFLD in lean individuals is well documented, the clinical implications of having lean NAFLD are less clear. Data on long-term mortality outcomes are quite scarce, including of HCC as an outcome In some liver biopsy studies, a significant number of these lean patients with NAFLD had NASH and advanced fibrosis. In a study with 49 months of follow-up, mortality rates were not different between obese and lean NAFLD patients, with cardiovascular events being the most important outcomes[81]. Overall, at 214 months of follow-up, NAFLD remained independently associated with increased risk of all-cause mortality (adjusted HR 1.54, 95%CI 1.25–1.89)[81].

Alcohol-related liver disease

Metabolic syndrome and alcohol consumption are the two important causes of chronic liver disease and, probably, one condition is frequently predominant, with the other acting as a cofactor. Obesity plus alcohol can act synergistically to overload the risk of liver fibrosis, carcinogenesis and mortality; and the genetic profile can also influence the disease progression. On the other side, while abstinence prevent disease progression and complications in patients with alcohol-related liver disease (ALD), there are debates about free mild /moderate alcohol consumption in NAFLD patients[82].

ALD or NAFL have common pathways to steatosis through an imbalance in fatty acid synthesis and β-oxidation. In NAFLD, steatosis is the consequence of lipid accumulation whereas in ALD, it is the consequence of direct ethanol toxicity in hepatocytes[82]. Adipose tissue generates pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) and produces adipokines (leptin, adiponectin). Leptin has pro-inflammatory activity that normally prevents the accumulation of lipids in the liver, reducing the SREBP-1c expression; and adiponectin has anti-inflammatory effects by inhibiting the release of TNF-α and IL-6, secreting anti-inflammatory cytokines and blocking NF-kB activation and improving hepatic and peripheral insulin resistance. When the level of leptin increases, its profibrogenic role is predominant, hepatic stellate cells are activated by the sonic hedgehog and mTOR pathways[82]. In obese individuals, there is a reduction in adiponectin and an increase in leptin levels, resulting in steatosis, inflammation and fibrogenesis[82]. However, chronic exposure to ethanol induces CYP2E1 in adipose tissue, resulting in and oxidative stress, leading to dysregulation of adipokines and progression of ALD[82].

Dysbiosis in both ALD and NAFLD leads to gut barrier dysfunction and increased intestinal permeability with increased levels of endotoxins and enhancing pathogen-associated molecular pattern (PAMP)-induced liver inflammation. The chronic alcohol intake results in increased intestinal TNF-α production causing disruption of intestinal tight junctions and intestinal barrier dysfunction[83]. NAFLD leads to endogenous production of alcohol resulting in the same intestinal barrier dysfunction[83].

Data have shown a specific microbiota signature related to NAFLD liver injury. Among patients with biopsy-proven NAFLD, a reduction in *Firmicutes* and an increased level of *Proteobacteria* (including *E. coli*) were observed in patients with advanced liver fibrosis; while the *Ruminococcus obeum* was in lower levels in advanced fibrosis than in mild/moderate NAFLD[84].

In the ALD there is colonic dysbiosis with a lower proportion of *Bacteroidetes* and a higher proportion of *Proteobacteria* if compared with non-ALD/non-drinkers. Along with the bacterial dysbiosis, recent data found changes in the abundance and composition of the faecal microbiome (commensal fungi) in mice after chronic alcohol administration and in alcohol-dependent patients. Daily alcohol consumption for 10 weeks modifies colonic mucosa-associated with bacterial microbiota composition in rats[84,85].

A variant rs72613567: TA in *HSD17B13*, encoding the hepatic lipid droplet protein hydroxysteroid 17-beta dehydrogenase, modulates liver inflammation and fibrosis but does not have a significant role in lipid accumulation in the liver[86]. This polymorphism was associated with reduced risk of alcoholic cirrhosis by 42% among heterozygotes and by 73% among homozygotes; the risk of NALFD-cirrhosis was reduced by 26% among heterozygotes and by 49% among homozygotes.98 It seems that HSD17B13[86]. The most of data about ALD or NAFLD genetic polymorphisms showed the critical role of factors associated with lipid metabolism in the liver, from the early stages to HCC[86].

CONCLUSION

Cirrhosis in patients with NAFLD/NASH represents the most significant risk for HCC. Among non-cirrhotic patients with NAFLD, variables such as male gender, type 2 diabetes, and smoking were associated with an increased risk of HCC. It is important to carry out studies that identify, in addition to risk factors, screening strategies for early detection and prevention of HCC in patients with NAFLD, especially considering the increase in this condition worldwide.

Authors' contribution

Cavalcante LN participated in the project design, review, as well as writing of the manuscript. Dezan MGF performed search and reviewed papers and writing. Paz CLSL and Lyra AC contributed to the discussion and final review of the manuscript.

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Cavalcante LN, Dezan MGF, Paz CLSL, Lyra AC. Fatores de risco para carcinoma hepatocelular em pacientes com doença gordurosa não alcóolica do fígado. Arq Gastroenterol. 2022;59(4):540-8.

RESUMO – A doença metabólica e doença hepática gordurosa metabólica estão aumentando a prevalência mundial e, portanto, espera-se um número maior de carcinoma hepatocelular (CHC) relacionado à doença hepática gordurosa não alcóolica (DHGNA) nos próximos anos. Esta revisão descreve os fatores de risco associados ao CHC em pacientes com DHGNA. A presença de cirrose hepática é a preponderante. Sexo masculino, variantes do gene *PNPLA3*, diabetes e obesidade também parecem predispor ao desenvolvimento de CHC, mesmo em indivíduos não cirróticos. Até agora, modificações significativas no estilo de vida, incluindo controle glicêmico e tratamento da obesidade, são terapias eficazes para DHGNA/ Esteatohepatite não-alcoolica e, portanto, provavelmente, também para CHC. Alguns medicamentos que propunham-se diminuir a atividade inflamatória e fibrose, bem como a obesidade, foram estudados. Outros dados sugeriram a possibilidade de quimioprevenção do CHC. Até o momento, no entanto, não há evidências definitivas para o uso rotineiro desses medicamentos. Esperamos, no futuro, poder traçar o perfil de pacientes com maior risco de DHGNA-CHC e traçar estratégias para diagnóstico precoce e prevenção.

Palavras-chave – Carcinoma hepatocelular; esteatohepatite não alcoólica; doença hepática gordurosa não alcoólica; DHGNA.

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