The Influence of Metabolic Syndrome on the Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B Infection in Mainland China

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Abstract

Background: The association between metabolic syndrome (MS), both in terms of its components and as a whole, and the risk of hepatocellular carcinoma (HCC) in subjects with hepatitis B virus (HBV) infection remains unclear, especially in mainland China.

Methods: We prospectively included 6,564 individuals with HBV infection from an initial cohort of 105,397 civil servants. The multivariate-adjusted HR and 95% confidence interval (95% CI) were evaluated using Cox proportional hazards models to explore the potential connection between HCC risk and MS. Cumulative incidences were plotted using Kaplan–Meier curves.

Results: After a 45,668.0 person-year follow-up (76.0 ± 30.8 months) of 6,564 subjects who were seropositive for hepatitis B surface antigen, 89 incident HCC cases were identified. MS as a whole was independently associated with a 2-fold increased HCC risk (HR, 2.25; 95% CI, 1.41–3.60) after adjusting for age (in 1-year increments), gender, cigarette smoking, alcohol consumption, liver cirrhosis, and elevated aspartate aminotransferase levels (≥40 U/L). Subjects with three or more factors and those with one or two factors had adjusted HCC risks of 2.12-fold (95% CI, 1.16–3.89) and 1.28-fold (95% CI, 0.74–2.22), respectively, in comparison with those without any metabolic factors. Central obesity and type 2 diabetes were associated with significantly increased HCC risk, whereas this association was not observed in obese subjects (body mass index ≥30 kg/m²; 95% CI, 0.73–3.44).

Conclusions: MS as a whole, central obesity, and type 2 diabetes were independently associated with increased HCC risk in a population with HBV infection in mainland China. Impact: MS may be a risk factor for HCC.

Introduction

Hepatitis B or C virus (HBV/HCV) infections (1) are well-known risk factors for hepatocellular carcinoma (HCC), and epidemiologic research has identified a multitude of other factors, including heavy alcohol consumption (ref. 2; mainly in Europe and North America), obesity (3), type 2 diabetes (4, 5), and tobacco smoking (6).

Viral infection in HCC cases was commonly observed to be copresent with other etiologic factors of this malignancy (6–8). It has been reported that in areas with a high prevalence of hepatitis viral infections, 8.4% to 24.1% of HBV/HCV-related HCC cases presented with type 2 diabetes (7, 9–12), 19.6% to 41.7% with hypertension (11, 12), 5.4% to 23.4% with alcohol abuse (13), and 28.3% to 33.0% with smoking habits (4, 13). Previous studies have demonstrated synergistic effects between HCC infections and alcohol consumption (2), obesity and diabetes (7, 14). Fortunately, effective antiviral regimens for HCV infection are now available. Thus, the prevalence of HCC related to HCV is anticipated to decline. However, HBV-related HCC is projected to increase for several decades (1) because of the high prevalence of HBV infection and the prolonged latency of HCC development.

Few studies are available on the influence of metabolic factors on HCC risk in chronic hepatitis B (CHB) patients, and they have shown mixed results (4, 5, 9, 10, 15–19). Most were conducted in Western countries (3, 8, 20) and Taiwan (4, 15, 16, 18), and obesity and type 2 diabetes were the two major observed factors.

China has the largest population of HBV infections worldwide and is unfortunately one of the countries that are suffering from the overwhelming burden of HCC. It has been reported that nearly half of new HCC cases every year worldwide occur in China (21). In addition, China is also faced with the challenge of a growing prevalence of metabolic syndrome (MS; ref. 22), which comprises central obesity, raised fasting plasma glucose, hypertension, elevated triglycerides, and reduced high-density lipoprotein (HDL). Nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of MS, is present in up to 15% of community-based populations in China (23), along with a higher prevalence of HBV infection. However, no large cohort study with follow-up has explored the relationship between MS and HCC risk among CHB patients.
patients in mainland China until now, and several case–control studies have resulted in inconsistent conclusions (10, 11). In addition, HBV-related factors (antiviral therapy, HBeAg status) and other potential factors that may affect HCC risk were not always well adjusted. Moreover, most previous research studies focused on diabetes and obesity, but insulin resistance (IR), regarded as the key process through which MS promotes carcinogenesis, is observed in various types of metabolic factors and can even occur without overt metabolic factors (24). Thus, we have conducted a prospective cohort study to explore the potential link between HCC risk and MS among individuals with chronic HBV infection in mainland China.

Materials and Methods

Study design

West China Hospital Physical Examination Center provides comprehensive physical examinations to an average of more than 110,000 people/year. More than 240,000 city residents received liver-related physical examinations between 2007 and 2015 in this center. The social backgrounds of the participants at our medical center vary, and the populations have different levels of income, education, and health awareness, which can affect their fitness status in numerous ways. Thus, we studied city civil servants who had access to free routine physical examinations covered by the government. This special subset of the population was assumed to have a relatively high education level (at least high school). Because highly educated populations are more likely to have healthier diets and better lifestyles, focusing on the civil servants population was expected to help reduce the interference of mixed factors mainly related to lifestyle. In addition, comprehensive clinical data are available for these persons, and follow-ups are easier to conduct. The study was approved by the hospital ethics committee, and written-informed consent for participation was obtained from each individual included in the study.

Detailed demographic data, medical history, and lifestyle habits were collected at enrolment, including (but not limited to) gender, working affiliation, cigarette smoking, and alcohol consumption. Body mass index (BMI) and waist circumference were measured by experienced nurses, and obesity was defined as BMI ≥ 30 kg/m² according to Chinese criteria reported by World Health Organization (WHO; ref. 25). The serum status of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), liver function, metabolic factors, and alpha fetoprotein (AFP) and abdominal ultrasound at baseline were tested in all participants. For all subjects, annual tests for total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), metabolic factors, AFP, and abdominal ultrasound were provided during follow-up. Total bilirubin, AST, and ALT were detected by electrochemiluminescence immunoassay using a Roche Cobas modular p800 automatic biochemical analyzer, whereas AFP was detected by electrochemiluminescence immunoassay using a Roche Elecsys modular analytics immunoassay analyzer. For any subject with a positive finding for AFP level ≥100 ng/mL or abdominal ultrasound at enrolment or at follow-up, enhanced contrast ultrasound or CT/MRI was conducted immediately. Three monthly tests for AFP, liver function, and abdominal ultrasound were offered to participants over 40 years old or those found to have hepatic dysfunction, elevated AFP, liver cirrhosis, or a benign or unidentified liver mass.

Study cohort

HBV infection status was tested in 105,397 subjects who received a physical examination at our center between 2007 and 2015, and 7,644 individuals were serum positive for HBsAg (7.25%) and were consecutively included in the cohort. Serological markers of HBV and anti-HCV antibodies were detected by electrochemiluminescence immunoassay using a Roche Elecsys modular analytics immunoassay analyzer. Because antiviral therapy and HBV DNA levels are well-characterized confounders for HCC risk, individuals with HBV DNA copy >10⁵/mL or have a history of antiviral therapy (294 individuals) were excluded from the cohort and accepted further management in the infectious disease department. Participants with the following characteristics were also removed from the cohort: individuals infected with HCV (20) and/or human immunodeficiency virus (13 individuals), HBV DNA copy number >10⁵/mL or undergoing anti-HBV therapy (294 individuals), or diagnosed with type 1 diabetes (1 individual), or a malignancy other than HCC (18 individuals). Eight HCC cases identified at enrolment, two with MS and six without MS, were also excluded from the final analysis. Ultimately, a cohort of 4,454 men and 2,110 women (Supplementary Fig. S1) who were HBsAg positive was formed after removing 746 participants who lacked data on metabolic factors.

Ascertainment of MS

The criteria outlined by the International Diabetes Federation (IDF) were used in this study. MS, defined by the IDF (22), the WHO (26), and the National Cholesterol Education Program (27), consists of complex physiologic biochemical and clinical factors that are not well understood and are now widely accepted that MS is mainly caused by central obesity and IR. Although IR is necessary for diagnosing MS defined by the WHO, the IDF dropped the requirement for IR from the diagnosis, but made the central obesity as a necessary criterion. Central obesity (22) is determined by a measurement of waist circumference (Chinese population, a man/woman ≥90/80 cm) or a BMI ≥ 30 kg/m². In addition, at least two of the following metabolic factors are required to complete the definition: (i) elevated triglycerides (≥1.7 mmol/L); (ii) reduced HDL (<1.03 mmol/L in males and <1.29 mmol/L in females); (iii) elevated blood pressure (BP, systolic BP ≥130 or diastolic BP ≥85 mm Hg); or (iv) elevated fasting plasma glucose (≥5.6 mmol/L). Individuals who were previously diagnosed with corresponding metabolic disease or are currently receiving specific treatments, regardless of their status at baseline, were considered to have the metabolic factor [e.g., a person diagnosed with type 2 diabetes mellitus (DM) or under insulin therapy is considered to have elevated fasting plasma glucose].

Metabolic factors other than MS as a whole and lifestyle factors

Subjects who met any of the following criteria were defined as having type 2 DM (17): (i) fasting glucose level ≥7.0 mmol/L, or positive oral glucose tolerance test (blood glucose level measured 2 hours post load of 75 g of glucose ≥11.1 mmol/L); (ii) glycosylated hemoglobin (HbA1c) ≥6.5%; or (iii) self-reported DM or receiving specific treatment for DM. Impaired fasting glucose (IFG; ref. 4) was identified as a fasting glucose level ≥6.0 mmol/L, and an oral glucose tolerance test was strongly recommended. Poorly controlled diabetes was defined with an HbA1c level higher than 7.0% (28). The definition of lipid factors for elevated triglycerides and reduced HDL is consistent with the
described in MS identification. A fasting serum total cholesterol of at least 6.2 mmol/L (240 mg/dL) was defined as hypercholesterolemia, and low-density lipoprotein (LDL) level was divided into high (≥4.10 mmol/L) and normal (<4.10 mmol/L) categories. Serum levels of AST and ALT were split into two categories with cutoffs at 40 U/L and 50 U/L, respectively. Smoking status was defined as current smoker, quitting, or nonsmoker. Alcohol consumption of more than 80 g/d for >3 times a week was defined as habitual alcohol consumption. Liver cirrhosis was determined by abdominal ultrasound, enhanced CT/MRI scan, or pathologic result.

Identification of HCC cases

HCC cases that were previously diagnosed and newly diagnosed at enrolment were excluded from the study. Patients who were diagnosed as having HCC within 3 months of follow-up were also excluded from the final cohort. Individuals with positive findings on abdominal ultrasound or an elevated level of AFP received an enhanced contrast ultrasound or CT/MRI. HCC cases were confirmed with one or more of the following criteria: (i) an AFP level higher than 400 ng/mL plus at least one positive finding in an imaging study (ultrasonography, enhanced CT, or MRI); (ii) an observed lesion with the consideration of HCC in two or more different imaging studies; or (iii) pathologic confirmation if a surgical resection or a biopsy was performed.

Statistical analysis

The person-time for each participant was computed from the date of enrolment to the date of HCC identification, death, or the end of follow-up (April 20, 2017), whichever came first. Analysis of variance and $\chi^2$ tests were used for continuous and categorical data, respectively, to compare the baseline characteristics of subjects with MS versus those without MS. Cox proportional hazards models were used to evaluate the effects of various risk factors on the risk of developing HCC, and HRs with corresponding 95% confidence intervals (CI) were estimated. Potential and significant variables determined in the univariate analysis were further entered in the multivariate analysis using Cox proportional hazards models. Type 2 diabetes, the five metabolic factors of MS, and diabetes were not entered into the multivariate-adjusted models when testing for the association between MS and the risk of HCC due to high variability in disease incidence and lack of significant effects on liver cirrhosis, were plotted using Kaplan–Meier curves, and differences between strata were determined by the Mantel–Cox test. Statistical analyses were performed using SPSS version 20.0 (IBM, Inc.).

Results

During the 45,668.0 person-year follow-up (average of 76.0 ± 30.8 months each) of 6,564 subjects who were seropositive for HBeAg, 89 incident HCC cases were identified; 45 of these cases were verified with postoperative histological pathology, and 1 was verified with biopsy. The whole cohort included 4,454 (67.9%) males and had an average age of 45.4 (±11.0) years; 780 individuals had cirrhosis (11.9%), 400 had diabetes (6.1%, including 194 self-reported DM), and 756 had identified MS (11.5%).

Baseline characteristics in subjects with or without MS

Subjects with MS were older ($P < 0.001$) and had a higher proportion of males ($P < 0.001$), type 2 DM ($P < 0.001$), elevated AST ($P < 0.001$), ALT ($P < 0.001$), and fatty liver ($P < 0.001$). A higher proportion of subjects without MS were HBeAg positive than those with MS. No significant differences were found for liver cirrhosis, total bilirubin, duration of DM longer than 5 years, and period of follow-up between the two groups. Table 1 compares the baseline characteristics between subjects with and without MS.

Univariate and multivariate analyses to detect risk factors of HCC

Among the whole cohort of 6,564 subjects, increased risk of HCC was related to male gender, age (in 1-year increments), MS, obesity, habitual alcohol consumption, liver cirrhosis, type 2 DM, and elevated levels of AST and ALT. Liver cirrhosis had the highest HR (14.70, 95% CI, 9.4–22.82) of all risk factors that had a positive relationship with HCC risk in univariate analysis, followed by type 2 DM (HR, 5.18; 95% CI, 3.2–8.38). A dose-response effect was observed in the association between increasing age and increased HCC risk, resulting in a 4.7% increased risk for each year. Both current smokers and those who had quit smoking were associated with nonsignificant higher HCC risks of 1.45-fold ($P = 0.95$) and 2.03-fold ($P = 0.13$), respectively, similarly with being seropositive for HBeAg and fatty liver disease. Neither elevated total cholesterol nor LDL was observed to have significant influence on HCC risk, with HRs of 0.46 ($P = 0.28$) and 0.62 ($P = 0.51$), respectively.

In the multivariate analysis model, older age (in 1-year increment), MS, habitual alcohol consumption, elevated AST ($≥40$ U/L), and cirrhosis were statistically significant risk factors after adjusting for each other and additional variables.
Cirrhosis maintained the strongest association with HCC risk with an HR of 11.00 (95% CI, 6.85–17.54) and was followed by MS (HR, 2.25; 95% CI, 1.41–3.60). Approximately 2-fold HCC risks were observed for habitual alcohol consumption (HR, 1.84; 95% CI, 1.08–3.13) and elevated AST level (HR, 1.95; 95% CI, 1.22–3.11). HCC risk increased by 2.1% in each 1-year increment (95% CI, 1.05–1.63).

Each individual metabolic factor and number of MS factors on HCC risk

In the analysis of each individual metabolic factor (five components of MS, obesity, total cholesterol, and LDL), central obesity and elevated fasting plasma glucose (≥5.6 mmol/L) were significantly associated with increased HCC risk after adjusting for sex, age (in 1-year increments), cigarette smoking, alcohol consumption, cirrhosis, and elevated AST levels. Higher HR was obtained in subjects with elevated plasma glucose (HR, 1.85; 95% CI, 1.18–2.89), followed by central obesity (HR, 1.73; 95% CI, 1.13–2.64). Individuals with elevated triglycerides (HR, 0.67; 95% CI, 0.39–1.16) or elevated total cholesterol (HR, 0.49; 95% CI, 0.12–2.00) were observed to have similar HCC risk to those with normal triglycerides or total cholesterol. In addition, no significant relationship was found between HCC risk and reduced HDL (P = 0.98), hypertension (P = 0.86), obesity (P = 0.24), or elevated LDL (P = 0.77). MS as a whole and DM were not entered into the model because of their significant correlation (contingency coefficient: 0.379, P < 0.001) with each metabolic factor. After adjustment, subjects with three or more factors and those with one or two factors had increased HCC risks of 2.12-fold (95% CI, 1.16–3.89) and 1.28-fold (95% CI, 0.73–2.22), respectively, in comparison with those without any factors. The details are described in Table 3.

**Fasting plasma glucose factor and increased HCC risk**

We conducted a multivariate regression model (Table 4) to further analyze the association between HCC risk and the status of plasma glucose factors. DM was associated with a 2.26-fold increased HCC risk (95% CI, 1.34–3.82) relative to subjects with normal plasma glucose (<5.6 mmol/L), whereas no significant difference was observed in subjects with IFG (HR, 0.96; 95% CI, 0.43–2.14) after adjusting for other factors. In subjects with poorly controlled DM, a 4.65-fold increased HCC risk (95% CI, 2.39–9.03) was observed, whereas well-controlled DM was associated with a nonsignificant higher risk of HCC (HR, 1.51; 95% CI, 0.78–2.92) relative to subjects without DM. No difference in HCC risk was found between subjects diagnosed with more than 5 years of DM and those with shorter durations of DM.

**Cumulative incidence of HCC in subjects with or without MS**

In the whole cohort, 756 individuals diagnosed with MS were found to have higher incidence of HCC in comparison with 5,808 MS-free individuals (10-year cumulative incidence 4.8% vs. 1.9%, HR: 3.13, P < 0.01, Fig. 1A). Subjects with 3 or more metabolic factors (HR: 3.66, P < 0.01) and those with 1 or 2 factors (HR: 1.73, P = 0.037) had increased incidences than subjects without any factor (10-year cumulative incidences of 4.0%, and 2.6% vs. 1.4%, Fig. 1B). Figure 2 shows the relative cumulative incidence

**Table 2. Univariate and multivariate-adjusted HRs of incident HCC in relation to potential risk factors**

| Variable             | HR (95% CI) | P                      | HR* (95% CI) | P                      |
|----------------------|-------------|------------------------|--------------|------------------------|
| Male gender          | 1.85 (1.00–3.10) | 0.020 | 1.07 (0.59–1.94) | 0.821                 |
| Age (y)              | 1.05 (1.00–1.06) | 0.000 | 1.02 (1.00–1.04) | 0.029                 |
| MS                   | 3.15 (1.98–4.95) | 0.000 | 2.25 (1.41–3.60) | 0.001                 |
| Obesity              | 2.36 (1.48–4.88) | 0.020 |                    |                       |
| Current smokers      | 1.45 (0.94–2.23) | 0.950 | 1.23 (0.74–2.02) | 0.429                 |
| Quitted              | 2.03 (0.81–5.11) | 0.131 | 0.97 (0.38–2.54) | 0.980                 |
| Alcohol consumption  | 1.0          |                     |              |                       |
| AST > 40 U/L         | 3.77 (2.40–5.93) | 0.000 | 1.95 (1.22–3.11) | 0.005                 |
| ALT > 50 U/L         | 1.84 (1.33–3.00) | 0.018 | 0.78 (0.42–1.44) | 0.422                 |
| HbA1c (−)            | 1.39 (0.64–3.01) | 0.407 |                    |                       |
| Fatty liver          | 1.62 (0.86–3.05) | 0.132 |                    |                       |

*Adjusted for sex, age in 1-year increments, habitual alcohol consumption, cigarette smoking, liver cirrhosis, and elevated level of AST (≥40 U/L).

**Table 3. Multivariate-adjusted HRs of HCC in relation to each single metabolic risk factor and metabolic factors sum**

| Variables               | Number of HCC cases/person-year | HR (95% CI) | P                      | HR* (95% CI) | P                      |
|-------------------------|---------------------------------|-------------|------------------------|--------------|------------------------|
| Central obesity         |                                 |             |                        |              |                        |
| Yes vs. no              | 40/1,980.0 vs. 49/33,560.5      | 2.29 (1.51–3.48) | <0.001 | 1.73 (1.13–2.64) | 0.012                 |
| Raised triglycerides    |                                 |             |                        |              |                        |
| Yes vs. no              | 17/10,369.0 vs. 72/33,171.5     | 0.80 (0.47–1.36) | 0.405 | 0.67 (0.39–1.16) | 0.352                 |
| Reduced HDL             |                                 |             |                        |              |                        |
| Yes vs. no              | 12/6,119.0 vs. 77/39,339.5      | 1.01 (0.55–1.85) | 0.992 | 0.99 (0.54–1.83) | 0.980                 |
| Raised glucose          |                                 |             |                        |              |                        |
| Yes vs. no              | 41/9,307.5 vs. 48/36,233.0      | 3.31 (2.18–5.03) | <0.001 | 1.85 (1.18–2.89) | 0.007                 |
| Hypertension            |                                 |             |                        |              |                        |
| Yes vs. no              | 32/11,240.5 vs. 57/34,307.5     | 1.7 (1.30–2.65) | 0.016 | 0.96 (0.60–1.55) | 0.862                 |
| MS factors ≥5           |                                 |             |                        |              |                        |
| MS factors = 2          | 27/6,654.0                      | 3.66 (2.09–6.41) | <0.001 | 2.12 (1.36–3.89) | 0.015                 |
| MS factors = 0          | 39/19,395.5                     | 1.73 (1.03–2.91) | 0.037 | 1.28 (0.74–2.22) | 0.377                 |
| Obesity                 |                                 |             |                        |              |                        |
| Yes vs. no              | 13/23,603.1                     | 1.0          | 1.0                    |              |                        |
| Raised cholesterol      |                                 |             |                        |              |                        |
| Yes vs. no              | 2/2176.5 vs. 87/45,392.0        | 2.56 (1.34–4.88) | 0.020 | 1.59 (0.73–3.44) | 0.242                 |
| Raised LDL              |                                 |             |                        |              |                        |
| Yes vs. no              | 2/1629.5 vs. 87/45,829.0        | 0.62 (0.15–2.53) | 0.507 | 0.81 (0.20–3.30) | 0.676                 |

*Adjusted for sex, age in 1-year increments, habitual alcohol consumption, cigarette smoking, liver cirrhosis, and elevated level of AST (≥40 U/L).

aCentral obesity: a man/woman with a waist circumference ≥90/80 cm, or with BMI ≥30 kg/m².
of HCC among subjects with MS versus those without MS, stratified by liver cirrhosis and alcohol consumption. In subjects without habitual alcohol consumption, the cumulative incidence of HCC significantly increased with the presence of MS (10-year cumulative incidence of 4.4% vs. 1.7%, HR: 3.19, \( P < 0.01 \)); however, the incidence of HCC did not increase with MS in subjects exposed to habitual alcohol consumption (10-year cumulative incidence of 6.7% vs. 4.4%, HR: 2.05, \( P = 0.119 \)).

In terms of liver cirrhosis, an increased cumulative incidence of HCC was observed in subjects with MS relative to those without MS, whether it was copresent with cirrhosis (10-year cumulative incidence of 23.5% vs. 10.6%, HR: 2.54, \( P < 0.01 \)) or not (10-year cumulative incidence of 1.4% vs. 0.7%, HR: 2.89, \( P < 0.01 \)). In summary, MS was significantly associated with an increased cumulative incidence of HCC independent of liver cirrhosis; however, this association was modified by habitual alcohol consumption.

### Discussion

To the best of our knowledge, this is the first prospective large-cohort study conducted in mainland China exploring the influence of metabolic factors on HCC risk in subjects with chronic HBV infection. Unlike many other countries in Asia, Western countries and Taiwan, where HBV, HCV, and alcohol consumption are common causes of HCC and cirrhosis (29–31), HBV infection has always been the dominant etiologic factor of HCC due to its long endemicity in mainland China. The proportion of HBV infection was 7.25% in the urban population used to select the study cohort, which is consistent with an earlier report (32). In contrast to previous studies in populations with mixed HBV and HCV infection statuses (4, 5, 15, 19, 33, 34), we studied subjects who were serum positive for HBsAg due to the special prevalence background in mainland China.

#### Table 4. Multivariate-adjusted HRs of HCC in relation to different status of plasma glucose and management of diabetes

| Variables                          | Number of HCC cases/person-year | Univariate HR (95% CI) | P    | Multivariate HR (95% CI) | P    |
|------------------------------------|----------------------------------|------------------------|------|--------------------------|------|
| DM vs. non-DM                      | 22/2,711.0                       | 5.18 (3.20–8.38)       | <0.001 | 2.28 (1.36–3.80)         | 0.002 |
| Fasting plasma glucose status      |                                  |                        |      |                          |      |
| DM (≥7.0 mmol/L)                   | 22/2,711.0                       | 5.53 (3.36–9.11)       | <0.001 | 2.26 (1.34–3.82)         | 0.002 |
| IFG (≥5.0 and <7.0 mmol/L)         | 7/2,760.5                        | 1.68 (0.77–3.71)       | 0.187 | 0.92 (0.43–2.14)         | 0.916 |
| Normal (<6.0 mmol/L)               | 60/40,069.0                      | 1.0                    |      |                          |      |
| Control of DM                      |                                  |                        |      |                          |      |
| Poorly                             | 11/679.0                         | 10.61 (5.60–20.08)     | <0.001 | 4.65 (2.39–9.03)         | <0.001 |
| Well                               | 1/2,032.0                        | 3.40 (1.80–6.43)       | <0.001 | 1.51 (0.78–2.92)         | 0.225 |
| Non-DM                             | 67/42,829.5                      | 1.0                    |      |                          |      |
| Duration of DM ≥5 years            |                                  |                        |      |                          |      |
| Yes vs. no                         | 15/1,679.0                       | 1.51 (0.61–3.73)       | 0.377 | 1.00 (0.39–2.58)         | 0.991 |

*Adjusted for sex, age in 1-year increments, habitual alcohol consumption, cigarette smoking, liver cirrhosis, and elevated level of AST (≥40 U/L).
In this prospective study, we demonstrated that MS as a whole was significantly associated with increased HCC risk in individuals with HBV infection in mainland China.

**Metabolic factors and HCC risk**

Several studies in the United States (8, 33–35), Europe (3, 36), Taiwan (4, 5, 15, 16, 18, 37), Hong Kong (38), and New Zealand (17) have examined the potential relationships between various metabolic factors and HCC risks, mostly focusing on type 2 DM and obesity. Diabetes (9, 11, 15–17, 39), or even prediabetes (40), has been observed to be a major metabolic factor related to an increased risk of HCC in individuals with HBV infections. This relationship was also detected in our study by analyzing 400 subjects (6.1%) with diabetes; these subjects had a 2.28-fold increased HCC risk relative to subjects without diabetes after adjusting for age, sex, cirrhosis, smoking, alcohol consumption, and AST levels. More specifically, IFG was not significantly associated with HCC risk, possibly due to the small number of subjects. In addition, patients with poorly controlled DM were significantly associated with a 4.65-fold increased HCC risk compared with those without DM in our study; however, a nonsignificant difference was observed between subjects with well-controlled DM (95% CI, 0.78–2.92) and those without diabetes.

Obesity is another crucial factor that has been studied over the past several years, and earlier studies have reported an association between obesity and an up to 4.0-fold increased risk (3, 41, 42) of HCC worldwide. However, in the current study, a nonsignificant (95% CI, 0.73–3.44) increased HCC risk was observed in obese subjects (BMI ≥ 30 kg/m²) in a multivariate regression model. However, subjects with central obesity (mostly diagnosed by enlarged waist circumference) had a significantly increased HCC risk (95% CI, 1.13–2.64) after adjusting for age, sex, cigarette smoking, alcohol consumption, elevated AST levels, and cirrhosis. This unique finding of different results for obese and centrally...

Figure 2.

Cumulative incidence of HCC in subjects with HBV infection. **A** and **B**, Incidence of HCC according to habitual alcohol consumption status. **C** and **D**, Incidence of HCC according to liver cirrhosis status. The P values were derived from the Mantel–Cox test.
obese individuals indicates that central obesity may better reflect central adiposity than obesity. In fact, central obesity, which corresponds to excess body fat in the abdomen, has been regarded as more indicative of MS than BMI (43, 44) and as a good predictor of hyperglycemia and hypertension (43). Kasmari and colleagues (35) demonstrated that hypertension is a risk factor for HCC regardless of cirrhosis, but we failed to detect this association between hypertension and HCC risk in our multivariate-adjusted model. Similarly, we did not identify any significant relationship between hypertriglyceridemia (95% CI, 0.39–1.16) and HCC risk, though it is expected to be positively related to HCC oncogenesis because of its close relationship with NAFLD and obesity. Some previous research studies found similar results (4, 16), but some others even indicated a protective effect of hyperlipidemia (15, 35) in HCC development. The exact mechanism responsible for the effect of serum lipids on HCC development remains unclear.

Effects of MS as a whole on HCC risk

To the best of our knowledge, the present report is the first to discuss MS, as defined by the IDF, as a whole factor in terms of its potential relationship to HCC risk in subjects with HBV infection. As expected, subjects with MS were older and had higher prevalence rates of central obesity, elevated fasting glucose, hypertension, elevated triglycerides, reduced HDL, cirrhosis, elevated levels of ALT/AST, and fatty liver disease than those without MS. Several studies have examined the potential association between a combination of various metabolic factors with their corresponding manifestations in the liver and HCC risk. However, none have evaluated the effect of this special syndrome as an independent factor on the development of HCC, and we are the first to identify MS as having a 2.25-fold increased HCC risk relative to individuals without MS after adjusting for age, sex, cigarette smoking, habitual alcohol consumption, elevated AST levels, and cirrhosis.

This significant association was independent of cirrhosis, whereas habitual alcohol consumption was a modifying factor. The association between MS and HCC risk was present in only nondrinkers, which supports NAFLD (20, 37) as an important pathway through which MS leads to a higher incidence of HCC. Note that the prevalence of MS in the current cohort was as high as 11.5%, which was nearly twice as high as that of type 2 diabetes (6.1%). A recent large-cohort study in Taiwan (5) indicated an association between aggregate metabolic risk factors and HCC incidence that resulted in substantially higher long-term risk in subjects with ≥3 metabolic risk factors than those with <3 factors. A similar result was found in our cohort in that subjects with 3 or more metabolic factors had a significantly higher HCC risk than those with fewer factors. Metabolic factors were further observed to increase HCC risk in a dose-response manner; the highest HR (2.12-fold) was for subjects with 3 or more factors in comparison with those without metabolic factors, whereas the HR was 1.28-fold for subjects with one or two factors.

To date, IR (5, 12) has been widely accepted as the common pathophysiological mechanism through which various metabolic risk factors lead to the oncogenesis of HCC. A sedentary lifestyle combined with excessive calorie intake leads to an imbalance between triglyceride deposition related to de novo lipogenesis and its removal via secretion or oxidation (45), mediated by IR, and contributes to lipid droplet accumulation and oxidative stress (46). Synergizing with hepatosteatosis, a necroinflammatory response (e.g., liver damage, inflammatory infiltrates, and fibrosis), causes nonalcoholic steatohepatitis (NASH), within which endoplasmic reticulum stress (47) and the accumulation of hepatic free cholesterol (48), a major lipotoxic molecule, are important characterizing factors. Tumor necrosis factor (TNF) is believed to be a key factor in the promotion of both NASH and its progression to steatohepatitic HCC by activating nuclear factor κB (NF-κB) signaling (47); thus, TNF may be a potential therapy target in the prevention of HCC.

Different factors supposedly influence and can be influenced by other factors. As a result, patients tend to acquire increasing risk factors if their current metabolic factors are not well managed. This finding was supported herein that there were more subjects with more than one metabolic factor than those with a single factor (1,999 vs. 1,726). In fact, IR can present without overt diabetes or obesity (12, 49), both of which are regarded as paramount risk factors for HCC. Therefore, better approaches were needed to systematically evaluate the effect of metabolic status on HCC development. Previous studies assessed IR with homeostasis model assessment (5, 12), which can be calculated with serum levels of insulin or C-peptide. However, serum insulin and C-peptide levels are not commonly tested during routine physical examinations in most subjects without overt diabetes or obesity. IDF-defined MS was much easier to assess; central obesity could be evaluated by simple waist circumference measurement, and other four components of MS were included in routine tests for HBV carriers in the Chinese population. Note HBV data on serum glucose, triglycerides, HDL, and BP were available for 6,508, 6,553, 6,553, and 6,558 out of 6,564 subjects, respectively. Consequently, the 2.25-fold increased HCC risk in relation to MS as a whole indicated that IDF-defined MS is a feasible and sensible predictor of HCC in Chinese individuals with HBV infections.

Effect of lifestyle factors

Our finding of a strong association between habitual alcohol consumption and a 1.84-fold increased HCC risk was consistent with previous studies (4, 15, 34). Even though the observed alcohol consumption rate (10.2%) was lower than that reported in Western populations (17, 34), alcohol abstinence is highly recommended for Chinese HBV carriers due to the strong relationship between alcohol consumption and increased HCC risk. Current smokers and those who quit smoking had an increased risk of HCC relative to subjects who never smoked, although neither result was significant in multivariate analysis, echoing previous findings (4, 13, 15). Two large population-based cohort studies in Taiwan (4, 15) detected a positive relationship between betel nut chewing and HCC risk; however, betel nuts are much less popular in mainland China; thus, the corresponding information was not recorded.

The major limitation of the current study involved the included cohort, in which all subjects were seropositive for HBsAg without the copresence of anti-HCV. Thus, a possible relationship between MS and HCC risk in individuals who are anti-HCV positive or free from viral hepatitis remains unclear in mainland China, and our findings may not necessarily be extended to other countries with different prevalence backgrounds of viral hepatitis. Because HBV infection is the predominant etiologic factor for HCC development in mainland China, the significant findings of the current study are expected to provide large benefits toward...
HCC prevention in China, which is also suffering an overwhelming burden due to the malignancy. Furthermore, the study cohort focused on government-employed civil servants (who have access to free routine physical examinations); thus, our results may not be applicable to the general HBV-infected population. Our study population choice may explain the relatively lower long-term incidence here compared with previously reported incidences for the general population (15, 50). In addition, family history of cancer or, more specifically, liver cancer was not analyzed because of the difficulty in obtaining this information. Currently, there is no cancer registry system in mainland China, and many patients could not provide information regarding the exact cause of death of their family members. The information on family history of cancer or liver cancer was therefore subject to recall bias and was confounded by other diseases. Given the long duration of follow-up, the subjects’ metabolic status or even lifestyle inevitably changed during the follow-up. Therefore, the influence of MS on HCC risk might be underestimated in subjects who had MS or habitual alcohol consumption at enrolment but had successful weight reduction or quit alcohol with medical intervention.

Conclusions

We prospectively assessed the effects of MS, both in terms of its components and as a whole, and lifestyle factors on HCC risk in subjects with HBV infection in mainland China. The significant association between MS and increased HCC risk indicates that MS is a feasible and sensitive predictor of this malignancy, given its higher prevalence over diabetes in the Chinese population with HBV infections. Therefore, subjects are expected to benefit from better management of metabolic factors and unhealthy lifestyles, including (but not limited to) weight reduction and cigarette cessation/alcohol abstinence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: Y. Tan, X. Zhang, J. Yang, T. Wen, H. Tang, L. Yan
Development of methodology: X. Zhang, W. Zhang, L. Tang, J. Yang
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Zhang, H. Yang, C. Li
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Tan, X. Zhang, L. Tang, H. Yang, K. Yan, J. Yang, H. Tang
Writing, review, and/or revision of the manuscript: Y. Tan, X. Zhang, W. Zhang, T. Wen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Zhang, J. Yang
Study supervision: L. Tang, L. Jiang, J. Yang, J. Yang, T. Wen, H. Tang, L. Yan

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