Epidemiological Analysis of Primary Hepatocellular Carcinoma Death and Its Mechanism of Promoting Cancer

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Abstract: Primary liver cancer (PLC) is the sixth most common cancer globally and the third leading cause of cancer-related deaths worldwide, ranking as the second leading cause of cancer deaths in China. Hepatocellular carcinoma (HCC) is the main histological type of PLC. Globally, 56% of PLC cases are due to Hepatitis B virus (HBV) infection, while 20% are due to Hepatitis C virus (HCV) infection. Chronic HBV infection is the main cause of PLC in East Asian countries including China, whereas HCV infection and non-alcoholic fatty liver disease are the main causes in most Western countries. This article analyzes the epidemiology of primary liver cancer deaths both domestically and internationally, as well as the impact of HBV infection on the survival of HCC patients, discussing the significant role of HBV infection in HCC. It explores the specific mechanisms through which HBV infection regulates TERT transcription via HBV-TERT integration, impacting the progression of HCC, and provides insights for clinical research on liver cancer.

Keywords: Liver Cancer; Epidemiology; Carcinogenesis Mechanisms

1. Introduction

China has the heaviest burden of liver cancer in the world. Primary liver cancer (PLC) is the sixth most common cancer globally and the third leading cause of cancer-related death, with approximately 9.06 million new cases and 830,000 deaths in 2020. The most common type of primary liver cancer worldwide is hepatocellular carcinoma (HCC), followed by cholangiocarcinoma[1]. The burden of PLC varies significantly by gender and geographic region, with chronic HBV infection being the main cause of HCC in Asian and African countries. In 2020, China had the highest age-standardized mortality rate for PLC in the world. China has the highest number of HCC patients, and despite the significant decrease in liver cancer incidence due to the widespread use of hepatitis B vaccines, the number of liver cancer deaths remains high[2].

HBV causes HCC through three main mechanisms: maintaining inflammation in chronic HBV infection, HBV self-variation, and integration of HBV sequences into the human body forming HBV integration mutations. Previous studies have provided ample evidence of key molecular events leading to HBV infection and HBV variation-induced carcinogenesis. However, there is still substantial knowledge gap in the mechanisms of HBV integration-induced carcinogenesis[3]. Through high-throughput sequencing data, the research team found that telomerase reverse transcriptase (TERT) encoding gene is the most common target of HBV integration, with approximately 26% of HBV-HCC cases having HBV inserted in the TERT promoter region. At the DNA level, HBV integration in the TERT promoter region is associated with increased TERT gene expression in cancer tissues ($P=3.89\times10^{-8}$); meanwhile, reverse insertion of the HBX segment in the upstream 3kb region of TERT also shows a trend of promoting TERT expression. HBV integration in the TERT promoter region (HBV-TERT) has been reported to be associated with TERT transcription levels and poor prognosis of HCC. However, the specific molecular mechanisms by which HBV-TERT promotes TERT transcription and the development of HCC are not yet clear[4]. Therefore, this study compares the epidemiology of PLC deaths in China and the United States in recent years, analyzes the impact of HBV infection on the prognosis of HCC patients, and explores the important role of HBV infection. And this study investigates the epidemiological analysis of primary liver cancer deaths and carcinogenesis mechanisms to provide reference for clinical research on liver cancer.

2. Epidemiological Analysis of Hepatocellular Carcinoma Death

2.1 Data Source

(1) The liver cancer mortality data in China from 2004 to 2020 is derived from the mortality surveillance dataset of the national disease monitoring system, with all original data released annually by the Chinese Center for Disease Control and Prevention. The national mortality surveillance system includes 605 monitoring points in 31 provinces (autonomous regions, municipalities directly under the central government), encompassing all liver cancer deaths with the ICD-10 code of C22. Based on the results of the first national economic census, the 31 provincial-level administrative regions are categorized into eastern (along the eastern coast), central (Heilongjiang, Jilin, Shanxi, Henan, Hubei, Anhui, Jiangxi, and Hunan), and western (other) regions. Furthermore, administrative areas below the county level (prefectures and counties) are designated as rural areas, while urban areas are designated as districts and above.

(2) The liver cancer mortality data in the United States from 1990 to 2020 is sourced from the Surveillance, Epidemiology, and End Results (SEER) database established in 1973 by the National Cancer Institute (NCI). The database consists of 18 cancer registries covering 28% of the US population. Primary tumors were identified using the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3), including all liver cancer deaths with the C220 code. Access to this database was facilitated through the SEER*Stat8.3.8 software to retrieve and download liver cancer mortality data.

(3) The 2020 Global Cancer Statistics report published by the International Agency for Research on Cancer was downloaded to obtain data on liver cancer mortality in China and the United States (https://gco.iarc.fr/today/).

(4) Download liver hepatocellular carcinoma cohort (LIHC cohort) liver cancer RNA-seq data and clinical information of liver cancer patients from The Cancer Genome Atlas (TCGA) database. The study group comprises 414 individuals from Asian, American, and African populations, including 364 liver hepatocellular carcinoma tissue samples and 50 adjacent tissue samples. The download link is https://tcgadata.nci.nih.gov/tcga/dataAccessMatrix.htm.

2.2 Statistical Analysis

2.2.1 Quality Control

All deceased cases in the National Mortality Surveillance System are reported online through the Cause of Death Reporting Information System of the Chinese Center for Disease Control and Prevention. The Chinese Center for Disease Control and Prevention reviews the data reported by each province, verifies and corrects any issues identified. SEER ensures quality assurance for all members in the cohort through systematic, standardized, and periodic data collection procedures to avoid surveillance bias. Case finding audits are conducted by qualified members of the SEER Registry under the guidance of the National Cancer Institute.

2.2.2 Statistical Analysis

(1) Joinpoint Regression Model

A joinpoint regression model (JRM) is used to describe the trends in liver cancer mortality in China from 2004 to 2020 and in the United States from 1990 to 2020. This is accomplished by comparing the annual percent change (APC) and the average annual percent change (AAPC) to 0. A change is considered statistically significant if the confidence intervals for both APC and AAPC do not contain 0.

(2) Survival Analysis

Total survival time is calculated using the life table method. Single-factor survival analysis is conducted using the log-rank test and Kaplan-Meier method, with KM curves generated. Variables selected from the single-factor analysis are included in a multifactor survival analysis. A Cox regression model is utilized for multifactor analysis to calculate the hazard ratio (HR) and 95% confidence interval (CI) for each variable. Variables with $P<0.05$ from single-factor analysis are further analyzed using stepwise regression for multifactor variable selection, and a Cox regression model is built using the results from all selected variables.

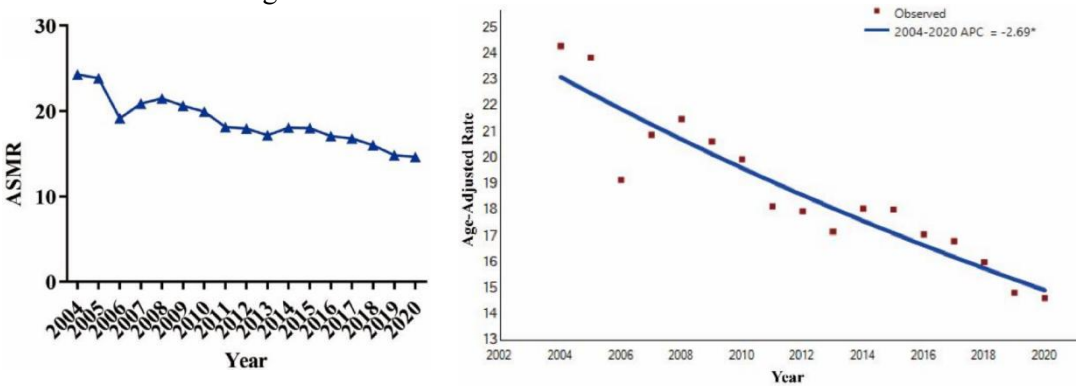
(3) Statistical Analysis

All data is processed and statistically analyzed using SPSS 22.0. Statistical indicators include age-standardized mortality rate (ASMR), APC, AAPC, median survival time, with a significance level set at $\alpha = 0.05$. The survival of HCC patients is assessed using the life table method, with a significance level of $\alpha = 0.05$.

2.3 Research Results

2.3.1 Analysis of Liver Cancer Mortality Rate in China from 2004 to 2020

Figure 1 shows the trend of liver cancer mortality rate in China from 2004 to 2020, with years on the x-axis and liver cancer incidence rate (per 100,000) on the y-axis. It presents a line chart illustrating the long-term variation of standardized mortality rate of liver cancer in China from 2004 to 2020 (see Figure 1(a)). It can be observed that the overall liver cancer Age-Standardized Mortality Rate (ASMR) in China exhibited a decreasing trend from 2004 to 2020 (AAPC=-2.7%, 95%CI: -3.2, -2.2, P<0.001) (Figure 1(b)). From 2004 to 2020, a total of 693,657 liver cancer deaths occurred in China, accounting for 16.50% of cancer-related deaths (n=4,204,344). In urban areas, there were 511,941 deaths, while in rural areas, there were 181,719 deaths. The number of male deaths (n=511,940) was approximately three times the number of female deaths (n=181,717). The liver cancer ASMR was calculated based on the 2000 Fifth National Census population in China.

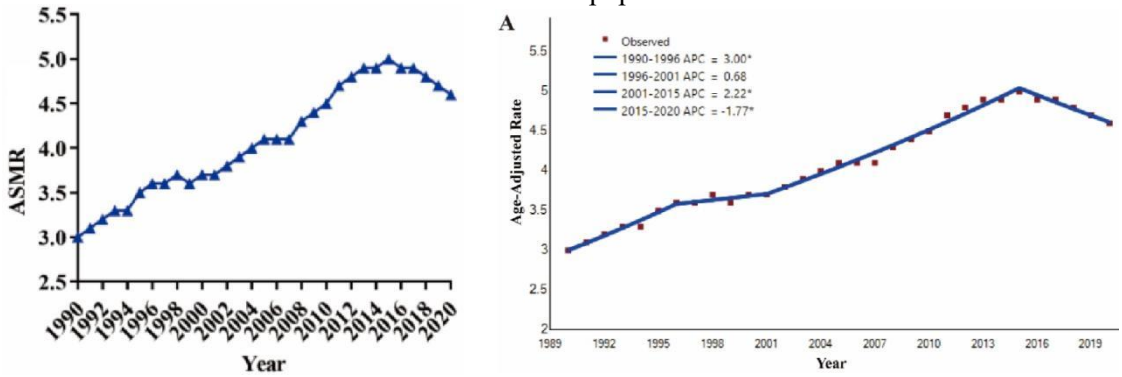


(a) (b)

Figure 1. Age-Standardized Mortality Rate (ASMR) of Primary Liver Cancer in China from 2004 to 2020

2.3.2 Analysis of Liver Cancer Mortality Rate in the United States from 1990 to 2020

Figure 2 illustrates the trend of liver cancer mortality rate in the United States from 1990 to 2020, with years on the x-axis and liver cancer incidence rate (per 100,000) on the y-axis. It depicts a line chart reflecting the long-term variation of standardized mortality rate of liver cancer in the United States from 1990 to 2020 (see Figure 2(a)). It can be observed that the overall liver cancer ASMR in the United States showed an upward trend from 1990 to 2020 (AAPC=1.4%, 95%CI: 1.2, 1.7, P<0.001), but from 2015 to 2020, there was a decline in liver cancer ASMR (APC=-1.8%, 95%CI: -2.6, -0.9, P<0.001) (Figure 2(b)). The liver cancer ASMR was calculated based on the 2000 U.S. population.



(a) (b)

Figure 2. Age-Standardized Mortality Rate (ASMR) of Liver Cancer in the United States from 1990 to 2020

2.3.3 Liver cancer mortality by age group in China and the United States in 2020

The Global cancer statistics report that in 2020, both the overall number of liver cancer deaths and age-specific death rates after the age of 20 in China were significantly higher than in the United States (Table 1).

Table 1. Death of Patients with Primary Liver Cancer

Age	Liver cancer deaths		Liver cancer ASMR	
	China	US	China	US
0-19	683	52	0.21	0.27
20-39	13017	274	2.7	0.28
40-59	151352	5247	32.2	5.3
>60	226100	25505	87.8	31.7
Total	391152	31078	17.2	4.7
Note: ASMR: Age-standardized rates (per 100000)				

2.4 Discussion

Primary liver cancer (PLC) is one of the leading causes of cancer-related deaths globally, with significant differences in liver cancer etiology across different regions. Key pathogenic factors of PLC include HBV infection, HCV infection, alcohol consumption, smoking, obesity, aflatoxins, non-alcoholic fatty liver disease, type II diabetes, and cirrhosis. This study evaluated the overall trends in liver cancer mortality from 2004 to 2020 in China and from 1990 to 2020 in the United States, comparing the situation of PLC deaths in China and the U.S. in 2020.

The study found that the age-standardized mortality rate (ASMR) of primary liver cancer in China showed a decreasing trend from 2004 to 2020, while in the United States, it exhibited an increasing trend from 1990 to 2020. The overall number of deaths and ASMR of PLC in China were significantly higher than those in the United States for all age groups over 20 years. On a global scale, East Asia has the highest incidence and mortality rates of hepatocellular carcinoma (HCC), but these rates have been declining in recent years. In contrast, the incidence and mortality rates of HCC in Europe and the United States are on the rise. Epidemiological monitoring indicates that HCC has been the fastest-growing cause of cancer-related deaths in the United States since the early 21st century. This trend may be attributed to the increasing rates of obesity and diabetes in the country due to economic development. Obesity and diabetes are risk factors for liver cancer, leading to an increase in both incidence and mortality rates. HBV and HCV infections are the main causes of HCC, with HBV infection accounting for 60% of HCC cases in Asia, while chronic HCV infection is the most common underlying liver disease among HCC patients in Europe and the United States.

Since the late 20th century, China has intensified efforts in liver cancer prevention and treatment. The widespread vaccination against hepatitis B since 1992 has significantly contributed to HBV prevention. Additionally, improvements in the healthcare system and economic development have facilitated early detection, diagnosis, and treatment of liver cancer patients, thereby reducing mortality rates. In the United States, the introduction of oral anti-HCV drug sofosbuvir in 2013 significantly decreased the risk of HCV-related liver cancer, potentially explaining the declining trend in liver cancer ASMR since 2015. The proportion of HBV-infected patients has gradually increased due to immigration, while non-alcoholic fatty liver disease associated with metabolic syndrome or diabetes in Western high-fat, high-sugar diet environments has become the fastest-growing cause of HCC.

This study found that in 2020, the ASMR for PLC patients aged 60 and above in the United States was 31.7, which is close to the ASMR for Chinese PLC patients aged 40-59 (32.2). Early studies indicate that evolution and integration of HBV genotypes during chronic infection directly promote the development of HCC, leading to HBV-related HCC occurring 10 years earlier than HCC caused by other etiologies, and being more aggressive. HBV infection drives the expression of alpha-fetoprotein through HBx protein, promoting the occurrence of HCC.

3. Mechanisms of Cancer Promotion Research

Telomerase enzyme activity and telomere length play a crucial role in the development of liver cancer. The expression of TERT is completely inhibited in adult liver cells, leading to continuous telomere shortening in these cells until senescence and apoptosis occur. During the process of liver cirrhosis, due to liver damage and constant cell turnover, telomeres become extremely short, triggering activation of DNA damage response, cell senescence, and

telomerase enzyme activation. Mutations in the TERT core promoter region lead to the formation of DNA sequences recognized by ETS-like transcription factors, thereby promoting their binding and becoming a key mechanism in the development of tumors by upregulating TERT[5].

TERT expression and telomere elongation can be detected in over 90% of HCC cases, correlating with tumor invasiveness and poor patient prognosis. The main mechanisms of telomerase activation in HCC include TERT core promoter mutations, TERT gene amplification, and integration of the HBV genome into the TERT promoter region. One of the highest frequency sites for HBV integration mutations is the TERT promoter region, which is rarely observed in non-tumor liver tissue. During HBV-induced hepatocellular carcinoma development, HBV evolves gradually by accumulating mutations that adapt to the inflammatory microenvironment and integrate into the human genome. Two proteins encoded by HBV, HBx and preS2, can respectively increase TERT expression and telomerase enzyme activity in liver cancer cells. Wild-type and C-terminally truncated HBx proteins have been shown to dose-dependently increase telomerase activity and TERT expression in different HBx-transfected cells[6].

Mutant and wild-type HBx and preS proteins can upregulate TERT promoter activity, although telomere length does not change significantly. Previous studies by the research group found that HBx and preS promote a malignant phenotype in HCC cells, leading to increased cell proliferation rates. Consequently, cell models overexpressing HBx and preS rapidly divide, increasing the rate of telomere attrition. Although TERT promoter activity increases, promoting telomere elongation, the rate of telomere elongation matches or even falls behind the rate of telomere attrition. Therefore, when measuring telomere length, there is no significant difference between the group overexpressing HBx and preS proteins and the control group. Mutant preS and HBx can upregulate TERT protein levels compared to wild-type preS and HBx at the mRNA level. This may be due to the direct interaction between mutant preS and HBx proteins with TERT protein to regulate its expression, rather than controlling TERT expression at the transcription level.

4. Conclusion

The overall Age-Standardized Mortality Rate (ASMR) of liver cancer in China and ASMR in each age group after the age of 20 are higher than in the United States, with a greater difference in ASMR between the two countries among patients over 40 years old. Our HCC cohort in China shows that the active replication level of HBV indicates an increased risk of death in HCC patients, while the TCGA LIHC cohort shows that HBV-infected patients have a higher risk of death. The prevention and treatment of HBV infection, as well as elucidating the HBV carcinogenesis mechanism, are of great significance for tertiary prevention of HCC. Regarding the carcinogenesis mechanism, HBV-TERT integration promotes TERT transcription by introducing new transcription factor binding sites. The full-length HBV sequence insert in the TERT promoter region may facilitate the maintenance of HBV infection and replication. TERT capture sequencing methods are consistent with HBV capture sequencing methods for detecting HBV-TERT integration efficiency and can be performed from peripheral blood, serving as a detection method for HBV-TERT integration in early clinical screening and prognostic prediction of HCC. The specific efficacy needs to be confirmed by subsequent large-scale epidemiological studies.

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