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Original Article

Protective effect of N-acetylcysteine against hepatocellular carcinoma in hepatitis B virus carriers

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Abstract: Hepatitis B virus (HBV) infection is a leading risk factor for hepatocellular carcinoma (HCC), contributing to cancer development through direct genomic integration and chronic inflammation. N-acetylcysteine (NAC), known for its antioxidant properties, is widely utilized in cancer prevention. However, clinical evidence regarding its protective effect against HCC in HBV carriers remains sparse. In this retrospective cohort study spanning 2008 to 2018, we utilized Taiwan's National Health Insurance Research Database (NHIRD) to include 1,061,174 chronic HBV carriers. Participants were stratified into NAC users and non-users using Propensity Score Matching. We assessed the incidence of HCC in both cohorts, examining the relationship between NAC usage duration and HCC incidence, and evaluating the dose-response effect. NAC users exhibited a significantly lower risk of developing HCC (adjusted hazard ratio [aHR]: 0.38; 95% confidence interval [CI]: 0.36-0.40; $P < 0.0001$). A dose-response relationship was evident, with higher cumulative defined daily doses (cDDDs) of NAC correlating with reduced HCC risk, revealing a significant trend ($P < 0.0001$). Notably, a daily NAC intensity of > 1.4 DDDs was associated with a decreased risk of HCC in HBV patients. Our results demonstrate that the use of NAC, in a dose-dependent manner, is intricately linked with a diminished incidence of HCC in individuals chronically infected with the HBV.

Keywords: Hepatocellular carcinoma, N-acetylcysteine, hepatitis B, reactive oxidase species, antioxidant agents

Introduction

Hepatocellular carcinoma (HCC) is the primary form of liver cancer and the fourth leading cause of cancer-related deaths globally [1]. While multiple risk factors exist, including exposure to liver-toxic agents, alcohol consumption, and metabolic-associated liver diseases, chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) remain the most significant contributors, accounting for approximately 80% of HCC cases [2]. In Eastern Asia and much of Africa, HBV is the predominant etiological agent for HCC. It is responsible for

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over half of HCC cases and ranks as the second leading cause of cancer deaths worldwide. Individuals with chronic HBV infection have a 10- to 25-fold higher risk of developing HCC compared to non-carriers [3]. In Taiwan, although the prevalence of HBV-related HCC in males decreased from 80% to 66% (and in females from 64% to 41%) over recent decades, HBV infection remains a principal risk factor for HCC in the region [4, 5]. HBV infection directly participates in hepatic transformation by initiating both common and virus-specific oncogenic pathways, alongside stimulating the host's immune response, leading to chronic liver necro-inflammation [6]. Among the complex interplay of HBV infection in the host, the role of HBV-induced mitochondrial reactive oxygen species (ROS) in promoting hepatocarcinogenesis has been extensively investigated [7, 8].

N-acetylcysteine (NAC), a mucolytic agent traditionally used for bronchial airway clearance, also serves as a precursor to L-cysteine and reduced glutathione [9]. Recent studies have expanded its therapeutic scope, revealing NAC's role as an antioxidant. Its clinical applications now extend beyond pulmonary diseases to include use as an antidote for acetaminophen overdose and in the prevention of contrast-induced nephropathy associated with imaging procedures. The hepatoprotective actions of NAC, attributed to its role as a precursor to glutathione (GSH), are believed to involve cytokine-mediated mechanisms in addition to glutathione replenishment [10]. Furthermore, NAC has garnered attention in the realm of oncology as a potential chemopreventive agent [11].

Numerous investigations have demonstrated the potential efficacy of NAC in cancer prevention and as an adjuvant in cancer treatment, particularly in the context of lung cancer [12] and triple-negative breast cancer [13]. A comprehensive literature review highlights pre-clinical studies illustrating NAC's capacity to mitigate reactive oxygen species (ROS) and inhibit hepatocarcinogenesis [14, 15]. Notably, clinical trials have indicated that intravenous administration of NAC, serving as a hepatic protector, reduces the incidence of post-embolization syndrome following trans-arterial chemoembolization in patients with HCC [16]. Despite these advancements, a notable gap in the existing data pertains to the protective effects of NAC use in individuals carrying the HBV against the development of HCC. This nationwide population-based study aims to analyze whether NAC exhibits a protective effect in preventing HCC development in HBV carriers and seeks to estimate the optimal dosage of NAC that yields the most robust protective effects.

Methods

Study population

Our cohort study, conducted from 2008 to 2018 using Taiwan's National Health Insurance Research Database (NHIRD), included Chronic Hepatitis B Carriers (HBV). This database, covering over 99% of Taiwan's population, provided encrypted patient data, including diagnoses, procedures, and prescriptions [17-21]. Linked to the Taiwan death registry, it enabled us to ascertain the patients' mortality status and causes of death, offering a comprehensive view for our analysis [17-21].

This study assessed HBV patients aged 18 or older from the NHIRD, excluding those with missing age data. NAC use was defined as 28 or more cumulative defined daily doses (cDDDs) following HBV diagnosis. The observation period started from the initiation of NAC treatment ≥ 28 cDDD (the index date) and continued until HCC diagnosis, death, or December 31, 2021. The case group comprised patients prescribed at least 28 cDDDs of NAC, while the control group had no NAC prescription. Follow-up lasted one year post-initial NAC use or cohort entry. This study aims to clarify the link between NAC use and HCC risk in HBV patients.

In our study examining HBV patients, we implemented specific exclusion criteria to maintain the integrity of the data. Exclusions applied to individuals who: (1) were diagnosed with HCC within one year following the index date; (2) lacked complete data regarding sex and age, or were below 18 years; (3) had a follow-up period shorter than one year; (4) had a prior diagnosis of any cancer type before joining the cohort; or (5) used NAC prior to their HBV diagnosis. These criteria were essential to ensure the reliability of our findings regarding the relationship between NAC use and HCC risk among HBV patients.

The Institutional Review Board (IRB) of the Tzu-Chi Medical Foundation granted approval for our study protocols (IRB Approval No. IRB109-015-B).

NAC exposure

NAC use was operationalized as the administration of a minimum of 28 cDDDs. NAC, a versatile agent in medicine, primarily serves as an antidote to acetaminophen toxicity by replenishing glutathione and neutralizing detrimental metabolites [10]. It is also commonly used as a mucolytic in treating respiratory disorders like chronic bronchitis, aiding in mucus clearance and respiratory function enhancement [22]. Considering the potential variability in NAC consumption over the study period, we accounted for NAC use as a time-varying covariate in our Cox model.

The cumulative NAC dose was computed by dividing the prescribed dose by the days' supply. We employed the World Health Organization's defined daily dose (DDD) standard to quantify NAC dosage, where the DDD represents the average maintenance dose per day for an adult's primary drug indication. To assess the impact of NAC daily intensity on HCC risk, we categorized usage into ≥ 1 DDD and < 1 DDD groups, with ≥ 1 DDD indicating substantial daily NAC use. The cDDDs were summed to verify a minimum intake of 28 cDDDs. NAC nonuse was classified as 0 cDDDs throughout the follow-up, excluding sporadic usage, while NAC use was defined as at least 28 cDDDs. We further stratified patients into four subgroups based on cDDD quartiles.

PSM and covariates

In our analysis, we controlled for potential confounders by including a range of covariates. We categorized study participants into four age groups at the index date: 18-30, 31-40, 41-50, and over 50 years. The index date for NAC users was the initiation of NAC at a minimum of 28 cDDDs. For matched non-users of NAC, the index date corresponded to the same variables' assessment date.

To ensure accuracy in our multivariate analysis, we avoided duplication in comorbidity adjustments. Comorbidities, identified within one year of the index date, were based on the International Classification of Diseases (ICD) codes. These were either from a primary inpatient diagnosis or from at least two outpatient visits within the year.

We utilized both the Ninth Revision, Clinical Modification (ICD-9-CM) and the Tenth Revision, Clinical Modification (ICD-10-CM) for coding. We employed a time-varying Cox proportional hazards model to examine the association between NAC use and HCC development, accounting for potential confounders. To robustly compare HCC risk between NAC users and nonusers, patient matching was based on propensity scores. Matching criteria included age, sex, income level, urbanization, Charlson Comorbidity Index (CCI) scores, existing comorbidities, and specific medication usage (anti-HBV treatment, statins, metformin, aspirin).

Continuous variables are reported as either means ± standard deviations or medians with interquartile ranges, as appropriate. To align patient groups more closely, we utilized the greedy matching technique: Propensity Score Matching (PSM) with a caliper width of 0.1, achieving a 1:1 match [23]. This approach involves pairing controls with comparable covariates considered essential by the researchers for effective confounder control.

Primary endpoints

The primary endpoint of our study was the incidence of HCC, verified by the certification records in the Catastrophic Illness Patient Registry [24].

Statistical analysis

We gathered patient characteristics as covariates, detailed in **Table 1**. Age was categorized in decade intervals. To compare baseline characteristics between NAC users and nonusers, we employed the chi-squared test for categorical variables, the t-test for continuous variables, and the Wilcoxon rank-sum test for median comparisons. The cohort entry date was established as the baseline.

To evaluate the relationship between NAC use and HCC risk, we computed incidence rates (IRs) and incidence rate ratios (IRRs), and estimated adjusted hazard ratios (aHRs) with 95% confidence intervals (CIs) using Cox regression

Table 1. Comparative characteristics of chronic hepatitis B carriers before and after propensity score matching, with and without N-acetylcysteine use

	Before PSM					After PSM				
	Never-NAC use N=978,235		NAC Use N=82,939		ASMD	Never-NAC use N=82,939		NAC Use N=82,939		ASMD
	N	%	N	%		N	%	N	%	
Age, years-old (mean ± SD)	41.60 ± 13.71		51.67 ± 15.73			50.79 ± 14.79		51.67 ± 15.73		
Age, median (IQR), years-old	40.00 (31.00, 51.00)		51.00 (40.00, 63.00)			51.00 (40.00, 61.00)		51.00 (40.00, 63.00)		
Age group, years					0.3363					0.0140
18-30	233,516	23.87%	8,192	9.88%		8,074	9.73%	8,192	9.88%	
31-40	256,632	26.23%	13,400	16.16%		13,139	15.84%	13,400	16.16%	
41-50	240,352	24.57%	18,183	21.92%		17,994	21.70%	18,183	21.92%	
> 50	247,735	25.32%	43,164	52.04%		43,732	52.73%	43,164	52.04%	
Sex					0.0507					0.0140
Female	421,995	43.14%	37,870	45.66%		38,447	46.36%	37,870	45.66%	
Male	556,240	56.86%	45,069	54.34%		44,492	53.64%	45,069	54.34%	
Income (NTD)					0.2011					0.0010
Low income	177,501	18.15%	18,869	22.75%		18,666	22.51%	18,869	22.75%	
≤ 20,000	372,325	38.06%	36,962	44.57%		36,827	44.40%	36,962	44.57%	
20,001-30,000	157,901	16.14%	10,776	12.99%		10,923	13.17%	10,776	12.99%	
30,001-45,000	157,483	16.10%	9,616	11.59%		9,642	11.63%	9,616	11.59%	
> 45,000	113,025	11.55%	6,716	8.10%		6,881	8.30%	6,716	8.10%	
Urbanization					0.1022					0.0060
Rural	236,050	24.13%	23,748	28.63%		23,525	28.36%	23,748	28.63%	
Urban	742,185	75.87%	59,191	71.37%		59,414	71.64%	59,191	71.37%	
CCI Scores										
Mean (SD)	1.34 ± 1.24		1.88 ± 1.60			1.77 ± 1.44		1.88 ± 1.60		
Median (Q1-Q3)	2.00 (0.00, 2.00)		2.00 (0.00, 3.00)			2.00 (0.00, 2.00)		2.00 (0.00, 3.00)		
CCI Scores					0.2663					0.0124
0	382,208	39.07%	22,118	26.67%		22,580	27.22%	22,118	26.67%	
≥ 1	596,027	60.93%	60,821	73.33%		60,359	72.78%	60,821	73.33%	

CCI										
Congestive Heart Failure	10,909	1.12%	3,786	4.56%	0.2366	3,294	3.97%	3,786	4.56%	0.0292
Dementia	1,986	0.20%	1,235	1.49%	0.1886	626	0.75%	1,235	1.49%	0.0704
Chronic Pulmonary Disease	70,853	7.24%	21,290	25.67%	0.5525	15,015	18.10%	21,290	25.67%	0.1839
Rheumatic Disease	13,625	1.39%	2,303	2.78%	0.0833	1,803	2.17%	2,303	2.78%	0.0393
Liver Disease	541,440	55.35%	48,102	58.00%	0.0044	51,121	61.64%	48,102	58.00%	0.0743
Diabetes with complications	13,295	1.36%	3,030	3.65%	0.1401	2,939	3.54%	3,030	3.65%	0.0059
Hemiplegia and Paraplegia	5,262	0.54%	1,204	1.45%	0.1088	845	1.02%	1,204	1.45%	0.0389
Renal Disease	15,950	1.63%	3,694	4.45%	0.1789	3,356	4.05%	3,694	4.45%	0.0198
AIDS	988	0.10%	83	0.10%	0.0896	79	0.10%	83	0.10%	0.0000
Cancer	25,627	2.62%	5,597	6.75%	0.1336	3,850	4.64%	5,597	6.75%	0.0911
Coexisting comorbidities										
Diabetes	83,473	8.53%	14,409	17.37%	0.2656	14,283	17.22%	14,409	17.37%	0.0040
Hypertension	150,101	15.34%	28,407	34.25%	0.4488	28,382	34.22%	28,407	34.25%	0.0006
Hyperlipidemia	129,783	13.27%	18,692	22.54%	0.2436	18,752	22.61%	18,692	22.54%	0.0017
Non-alcoholic steatohepatitis (NASH)	46,862	4.79%	5,918	7.14%	0.0993	5,583	6.73%	5,918	7.14%	0.0161
Alcohol-related liver diseases	21,873	2.24%	3,188	3.84%	0.0933	3,029	3.65%	3,188	3.84%	0.0100
CKD	9,057	0.93%	2,714	3.27%	0.1637	2,615	3.15%	2,714	3.27%	0.0068
Cholelithiasis	32,908	3.36%	4,995	6.02%	0.1261	4,691	5.66%	4,995	6.02%	0.0154
COPD	77,867	7.96%	24,060	29.01%	0.5634	23,837	28.74%	24,060	29.01%	0.0060
Pneumonia	29,231	2.99%	10,531	12.70%	0.3672	9,817	11.84%	10,531	12.70%	0.0262
Bronchitis	311,534	31.85%	41,003	49.44%	0.3640	41,085	49.54%	41,003	49.44%	0.0020
Pulmonary Cystic fibrosis	6	0.00%	3	0.00%	0.2130	3	0.00%	3	0.00%	0.0000
Myocardial infarction	3,317	0.34%	1,155	1.39%	0.1136	965	1.16%	1,155	1.39%	0.0205
Congestive heart failure	10,545	1.08%	4,356	5.25%	0.2399	3,898	4.70%	4,356	5.25%	0.0253
Cerebrovascular disease	29,763	3.04%	9,028	10.89%	0.3121	8,611	10.38%	9,028	10.89%	0.0165
Obesity	8,103	0.83%	1,010	1.22%	0.0387	920	1.11%	1,010	1.22%	0.0103
Ascites	4,440	0.45%	624	0.75%	0.0389	523	0.63%	624	0.75%	0.0145
Hepatic coma	2,255	0.23%	416	0.50%	0.0448	348	0.42%	416	0.50%	0.0118
Medication use										
Anti-HBV treatment	63,264	6.47%	7,743	9.34%	0.1065	7,655	9.23%	7,743	9.34%	0.0038
Statin	146,016	14.93%	23,042	27.78%	0.3175	23,673	28.54%	23,042	27.78%	0.0021
Metformin	93,003	9.51%	14,753	17.79%	0.2429	14,945	18.02%	14,753	17.79%	0.0060
Aspirin	132,783	13.57%	28,269	34.08%	0.4960	28,891	34.83%	28,269	34.08%	0.0158

NAC, cDDD										
Mean (SD)	0		143.16 ± 283.10			0		143.16 ± 283.10		
Median (q1, q3)	0		58.82 (38.02, 119.40)			0		58.82 (38.02, 119.40)		
NAC, cDDD										
Never use	978,235	100%	0	0.00%		82,939	100.00%	0	0.00%	
Q1	0	0.00%	20,581	24.81%		0	0.00%	20,581	24.81%	
Q2	0	0.00%	21,023	25.35%		0	0.00%	21,023	25.35%	
Q3	0	0.00%	20,538	24.76%		0	0.00%	20,538	24.76%	
Q4	0	0.00%	20,797	25.00%		0	0.00%	20,797	25.08%	
DDD (Daily density)										
Never use	978,235	100%	0	0.00%		82,939	100.00%	0	0.00%	
< 1	0	0.00%	38,799	46.78%		0	0.00%	38,799	46.78%	
≥ 1	0	0.00%	44,140	53.22%		0	0.00%	44,140	53.22%	
Mean (SD) follow-up time, year	7.57 ± 3.80		7.57 ± 3.78			6.72 ± 3.72		7.57 ± 3.78		
Median (IQR) follow-up time, year	7.64 (4.34, 10.91)		7.58 (4.36, 10.81)			6.47 (3.48, 9.60)		7.58 (4.36, 10.81)		
Primary outcome					*P*-value					*P*-value
HCC	33,863	3.46%	1,848	2.23%	< 0.0001	4,043	4.87%	1,848	2.23%	< 0.0001

Abbreviations: HCC, Hepatocellular Carcinoma; HBV, Hepatitis B Virus; NAC, N-Acetylcysteine; cDDD, Cumulative Defined Daily Doses; DDD, Defined Daily Dose; Q, Quartile; PSM, Propensity Score Matching; CCI, Charlson Comorbidity Index; NTD, New Taiwan Dollar; SD, Standard Deviation; IQR, Interquartile Range; NASH, Non-Alcoholic Steatohepatitis; COPD, Chronic Obstructive Pulmonary Disease; CKD, Chronic Kidney Disease; ASMD, Absolute Standardized Mean Difference; N, Number.

Table 2. Risk of hepatocellular carcinoma in propensity score matched chronic hepatitis B carriers: comparative analysis of N-acetylcysteine use, stratified by usage intensity and cumulative dosage

	Hepatocellular Carcinoma stroke risk					
	Crude HR (95% CI)	*P*-value	aHR* (95% CI)	*P*-value	aHR# (95% CI)	*P*-value
NAC (ref. Never-NAC use)	1.00 -	-	1.00 -	-	1.00 -	-
NAC use	0.41 (0.39, 0.43)	< 0.0001	0.38 (0.36, 0.40)	< 0.0001	0.39 (0.37, 0.42)	< 0.0001
cDDD of NAC (ref. Never-NAC use)	1.00 -	-	1.00 -	-	1.00 -	-
Q1	0.42 (0.38, 0.46)	< 0.0001	0.51 (0.46, 0.56)	< 0.0001	0.51 (0.46, 0.56)	< 0.0001
Q2	0.41 (0.37, 0.45)	< 0.0001	0.44 (0.40, 0.49)	< 0.0001	0.44 (0.4, 0.49)	< 0.0001
Q3	0.42 (0.39, 0.47)	< 0.0001	0.40 (0.36, 0.44)	< 0.0001	0.39 (0.36, 0.43)	< 0.0001
Q4	0.38 (0.34, 0.42)	< 0.0001	0.26 (0.23, 0.28)	< 0.0001	0.25 (0.23, 0.28)	< 0.0001
P for trend		< 0.0001		< 0.0001		< 0.0001
DDD of NAC (ref. Never-NAC use)	1.00 -	-	1.00 -	-	1.00 -	-
< 1	0.52 (0.48, 0.55)	< 0.0001	0.45 (0.42, 0.48)	< 0.0001	0.45 (0.42, 0.48)	< 0.0001
≥ 1	0.31 (0.29, 0.34)	< 0.0001	0.31 (0.29, 0.34)	< 0.0001	0.31 (0.29, 0.34)	< 0.0001
P for trend		< 0.0001		< 0.0001		< 0.0001

Abbreviations: NAC, N-Acetylcysteine; CI, Confidence interval; aHR, Adjusted hazard ratio; HR, hazard ratio; cDDDs, cumulative defined daily doses; DDD, defined daily doses; ref., reference group; Q, quartiles; N, Number. *The time-varying Cox model, which treats NAC use as a dynamic variable, was adjusted to account for several factors. These include age, sex, income levels, urbanization level, CCI Scores, other coexisting medical conditions, and the use of various medications. #The Fine and Gray method was adapted to estimate the hazard of HCC considering competing risks from death.

models. These adjustments accounted for factors including age, sex, income, urbanization, CCI Scores, existing comorbidities, and medication use, as listed in **Table 1**. Additionally, we employed time-varying Cox regression to analyze the impact of different cDDD levels of NAC and daily intensity (≥ 1 DDD or < 1 DDD) on HCC risk in HBV patients. The Fine and Gray methodology was utilized to account for ischemic stroke hazards, considering competing mortality risks. The Kaplan-Meier method estimated cumulative HCC incidence, with comparisons drawn using the log-rank test.

All statistical analyses were conducted using SAS for Windows (version 9.4; SAS Institute, Cary, NC). A two-sided *P*-value of less than 0.05 was deemed indicative of statistical significance.

Results

Demographic and clinical profile of the chronic hepatitis B cohort

In this investigation, we analyzed data from 1,061,174 HBV patients enrolled between 2008 and 2018, as detailed in **Table 1**. Prior to PSM, the NAC use group showed a higher prevalence of older individuals, lower income, more rural residency, increased CCI scores, and greater frequency of comorbidities such as diabetes, hypertension, hyperlipidemia, chronic kidney diseases, cholelithiasis, chronic obstructive pulmonary disease, pneumonia, bronchitis, pulmonary cystic fibrosis, myocardial infarction, congestive heart failure, and cerebrovascular disease. This group also had higher usage of concurrent medications including anti-HBV treatments, statins, metformin, and aspirin, compared to the never-NAC use group.

For comparative analysis, we conducted 1:1 matching, resulting in 82,939 patients in each group. The post-PSM age distribution was analogous in both groups (**Table 1**). Following PSM, variables such as age, sex, income levels, urbanization, CCI Scores, coexisting comorbidities, and medication use were statistically similar between the NAC user and never user groups. The crude incidence of HCC post-PSM was 2.23% in the NAC use group and 4.87% in the never-NAC use group (P < 0.0001).

HCC risk in HBV: NAC users vs. non-users

Post-PSM, no covariates listed in **Table 1** were significantly linked to HCC risk. The sole significant independent predictor identified was the use of NAC. The aHR for HCC in the NAC use group, relative to the never-NAC use group, was 0.38 (95% CI: 0.36-0.40, P < 0.0001), as shown in **Table 2**. When accounting for the competing risk of death, the aHR was 0.39 (95% CI:

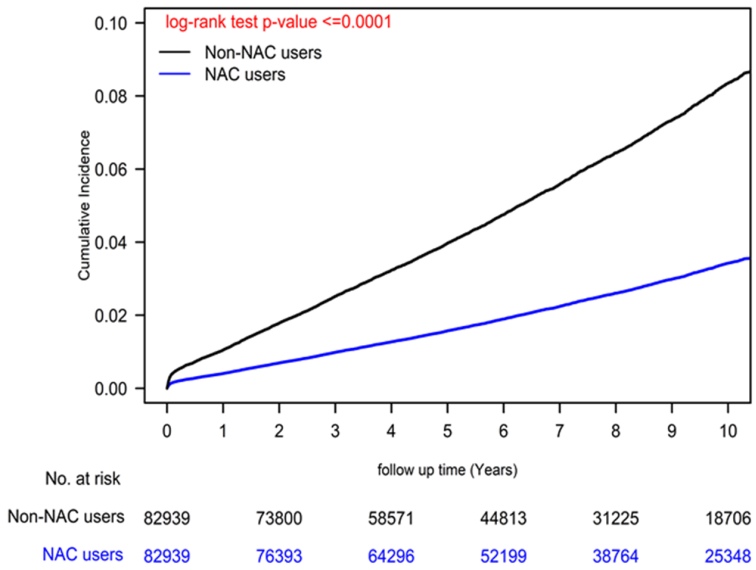

Figure 1. Kaplan-Meier analysis comparing the cumulative incidence of hepatocellular carcinoma in chronic hepatitis B patients with and without N-acetylcysteine use.

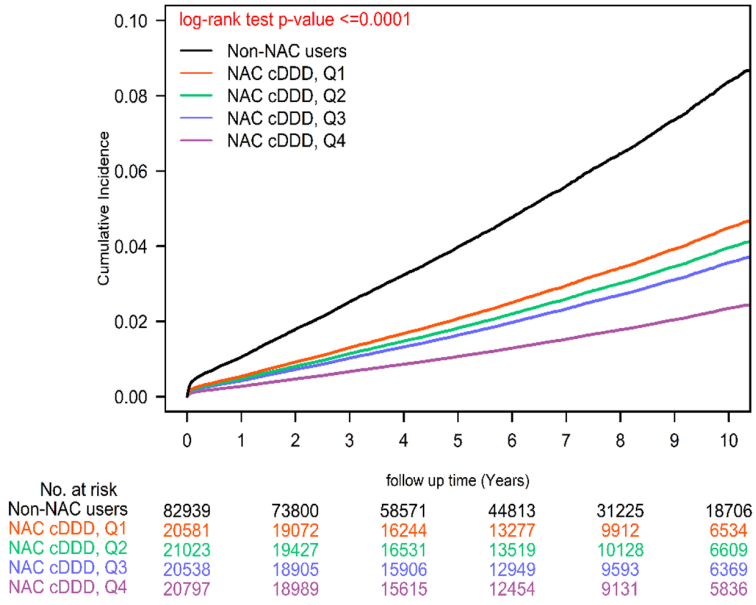

Figure 2. Kaplan-Meier survival curves depicting the cumulative incidence of hepatocellular carcinoma in chronic hepatitis B patients, categorized by various cDDD of N-acetylcysteine.

0.37-0.42) for NAC users versus non-users. Moreover, Kaplan-Meier curves depicting HCC cumulative incidence revealed a significantly higher rate in the never-NAC use group compared to the NAC use group (**Figure 1**, P < 0.0001).

Dose-response: NAC and HCC risk in HBV patients

We examined the dose-response relationship between NAC use and HCC risk by dividing cDDDs into four quartiles (Q1, Q2, Q3, and Q4), as shown in **Table 2**. The aHRs for HCC risk in these quartiles, in comparison to non-NAC users, were as follows: Q1, 0.51 (95% CI, 0.46-0.56); Q2, 0.44 (95% CI, 0.40-0.49); Q3, 0.40 (95% CI, 0.36-0.44); and Q4, 0.26 (95% CI, 0.23-0.28). A significant dose-response trend (P < 0.0001) was observed, indicating a reduction in HCC risk with increasing NAC use among HBV patients. This trend persisted even after adjusting for the competing risk of death. Kaplan-Meier curves also demonstrated significantly lower cumulative incidences of HCC in the NAC use groups, particularly in the higher quartiles (Q4 to Q1), compared to the non-NAC use group (**Figure 2**, P < 0.0001).

Impact of NAC daily dosing intensity on HCC risk in HBV patients

To evaluate the relationship between daily NAC dose intensity and HCC risk in HBV patients, we classified the DDD into two categories: DDD < 1 and DDD ≥ 1, as detailed in **Table 2**. The aHRs for HCC risk, relative to non-NAC users, were: DDD < 1, 0.45 (95% CI, 0.42-0.48); and DDD ≥ 1, 0.31 (95% CI, 0.29-0.34). A significant trend (P < 0.0001) was observed, indicating that increased daily NAC intensity corresponded with a decreased HCC risk in HBV patients. This trend remained significant even after accounting for the competing risk of death. Kaplan-Meier curves also showed a notably lower cumulative incidence

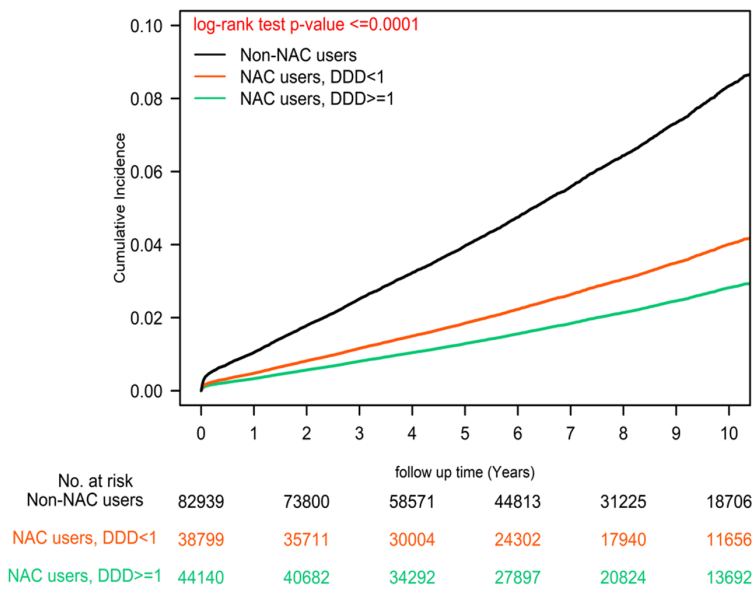

Figure 3. Kaplan-Meier curves illustrating the cumulative incidence of hepatocellular carcinoma among chronic hepatitis B carriers, segregated by different daily intensity levels (Defined Daily Dose, DDD) of N-acetylcysteine usage.

of HCC in the DDD ≥ 1 NAC group, followed by the DDD < 1 group, and then the never-NAC use group (**Figure 3**, P < 0.0001).

Additionally, Supplementary Figure 1 illustrates the relationship between NAC daily intensity (DDD) and the HCC hazard ratio in chronic hepatitis B carriers. It suggests that a daily dose of 1.40 DDD might be the minimal recommended dosage, as the HCC risk reduction capacity appears to plateau beyond NAC > 1.40 DDD, though there is still a gradual decrease in HCC risk with higher DDD of NAC use.

HCC incidence comparisons between NAC users and never-users

Table 3 delineates the association between NAC use and HCC development in our HBV cohort. The HCC incidence rate per 10,000 person-years was significantly lower in NAC users (29.45) compared to never-users (72.52). The incidence rate ratio (IRR) of HCC for NAC users, with a 95% CI, was 0.41 (0.38-0.43) relative to never-users.

A dose-response relationship between NAC use and reduced HCC risk was evident. IRRs for HCC in the quartiles of NAC use (Q1, Q2, Q3, Q4) were 0.42 (0.38-0.46), 0.41 (0.37-0.45), 0.40 (0.38-0.46), and 0.38 (0.34-0.42) respectively, compared to nonuse. Additionally, a correlation between daily dose density and HCC risk was observed. For NAC use at DDD < 1 and DDD ≥ 1, the IRRs were 0.52 (0.48-0.55) and 0.31 (0.29-0.34), respectively, compared to never-users.

Discussion

In our clinical investigation into the use of NAC and its potential to safeguard against HCC in HBV carriers, we unearthed several pivotal insights that could markedly influence both clinical practice and research. The cornerstone of our study is the emergent role of NAC, classically utilized as a mucolytic agent and as an antidote for acetaminophen toxicity, as a viable chemopreventive tool against HCC in individuals with HBV (see **Tables 2** and **3**; **Figures 2** and **3**). This reevaluation of NAC's clinical utility extends its therapeutic potential beyond its conventional use in respiratory conditions and acute liver failure. Critically, our data delineate a dose-response curve, illustrating that increased cumulative doses and intensified daily NAC administration correlate with a substantial diminution in HCC incidence among HBV patients (refer to **Tables 2** and **3**). This finding is particularly salient for clinicians, presenting a pragmatic chemoprevention strategy to curtail HCC risk in chronic HBV patients - a group notably susceptible to this malignancy. This is especially relevant considering the lifelong, costly anti-HBV therapies that are often not reimbursed under Taiwan's National Health Insurance, placing a significant burden on patients. Further, our analysis sheds light on the optimal NAC dosing required to maximize its protective effect against HCC (as illustrated in Supplementary Figure 1). This presents clinicians with an empirical basis to customize NAC treatment regimens, potentially heralding more individualized and efficacious preventive strategies against liver cancer in HBV carriers. Employing a comprehensive, decade-spanning dataset from Taiwan's NHIRD, our large-scale, nationwide population-based study confers

Table 3. Incidence rate ratios and hazard ratios for hepatocellular carcinoma in propensity score matched chronic hepatitis B carriers: detailed comparative analysis of N-acetylcysteine use, categorized by intensity of use and cumulative dosage

	Events	Person-years	IR (per 10,000 person-year)	IRR	95% CI for IRR	*P*
NAC use						
Never-NAC use	4,043	557,503.9	72.52	Ref.		
NAC use	1,848	627,439.9	29.45	0.41	(0.38, 0.43)	< 0.0001
NAC use (cDDD)						
Never-NAC use	4,043	557,503.9	72.52	Ref.		
NAC user dose, Q1	480	158,474.0	30.29	0.42	(0.38, 0.46)	< 0.0001
NAC user dose, Q2	476	161,316.8	29.51	0.41	(0.37, 0.45)	< 0.0001
NAC user dose, Q3	476	155,818.3	38.55	0.40	(0.38, 0.46)	< 0.0001
NAC user dose, Q4	416	151,830.8	27.40	0.38	(0.34, 0.42)	< 0.0001
NAC use (daily density, DDD)						
Never-NAC use	4,043	557,503.9	72.52	Ref.		
< 1	1,092	292,080.4	37.39	0.52	(0.48, 0.55)	< 0.0001
≥ 1	756	335,359.5	22.54	0.31	(0.29, 0.34)	< 0.0001

Abbreviations: NAC, N-Acetylcysteine; cDDD, cumulative defined daily dos; DDD, defined daily doses; IR, incidence rate; IRR, incidence rate ratio; Ref., reference; CI, confidence interval; Q, Quarter.

substantial validity to these findings. This extensive analysis not only bolsters the reliability of our results but also enhances their applicability to a broader patient population. Our study not only reaffirms the multifaceted application of NAC in clinical settings but also forges new pathways in oncological research, especially in the prophylaxis and management of HCC among HBV carriers. It beckons further exploration into the mechanistic underpinnings of NAC's protective properties and potentially lays the groundwork for groundbreaking therapeutic approaches to combat liver cancer in high-risk groups.

Chronic HBV infection, a known potent hepatocarcinogen, increases the lifetime risk of developing HCC by 10- to 25-fold compared to non-carriers [3]. The pathogenesis of HBV-related HCC involves both direct and indirect mechanisms, including HBV DNA integration into the host genome leading to genomic instability and insertional mutagenesis of various cancer-related genes [25]. Prolonged expression of viral regulatory proteins, such as HBx and altered versions of the preS/S envelope proteins, has been shown to disrupt cellular transcription and proliferation control, acting as carcinogenic factors [26, 27]. Particularly, the HBx protein induces ROS, creating a feedback loop that triggers carcinogenesis. Given this background, antioxidants like NAC, traditionally used as a mucolytic agent in respiratory diseases, could theoretically reduce ROS levels [28]. NAC's protective mechanisms are multifaceted, including the inhibition of mutagenic agents, reduction of ROS genotoxicity, and protection of DNA and nuclear enzymes [29-31]. Although NAC's role as a chemopreventive agent in upper airway malignancies has been considered, its efficacy in prolonging survival in primary lung cancer or head and neck cancer was not demonstrated in the EURSCAN randomized control trial (RCT) [32], possibly due to suboptimal dosing. It is hypothesized that NAC's efficacy in reducing HCC risk may be attributed to its potentiation of hepatic glutathione, enhancing liver detoxification processes, a mechanism possibly more effective in the liver than in lung or head and neck cancers [9, 10]. Recent studies have also highlighted NAC's role in reducing chemotherapy-induced neuropathy [13, 33] and its synergistic effects with chemotherapeutic agents in hepatopancreatic malignancies [34]. These findings open new avenues for the use of NAC in oncology, particularly in the prevention and adjunctive treatment of liver cancer, underscoring the need for further research to optimize dosing and application in various cancer types.

In the realm of liver disease management, NAC extends beyond its established role as an anti-

dote in acetaminophen poisoning, demonstrating substantial antioxidant effects through the augmentation of depleted glutathione levels in hepatocytes [15]. Clinically, NAC has shown a tendency to enhance liver function in conditions such as non-alcoholic fatty liver disease and alcoholic liver disease, as evidenced by studies [35, 36]. However, its application in the treatment of HBV infection has not been extensively explored. To our knowledge, this study represents the inaugural clinical evidence of NAC's protective role against malignant transformation in HBV carriers. A critical observation from our data is the importance of both the cumulative dosage and the daily intensity of NAC in diminishing the incidence of HCC and associated mortality risk. Particularly noteworthy is the finding that higher cDDDs of NAC correspond with a significantly lower risk of developing HCC (aHR=0.25, as shown in **Table 2** and **Figure 2**). This suggests that long-term NAC administration may confer enhanced HCC preventive benefits in the HBV carrier population. Additionally, our study identified a significant protective threshold for HCC incidence at a daily NAC dosage of 1.40 defined daily doses (DDDs), with a continued, albeit gradual, decrease in HCC risk at higher NAC dosages. This implicates a minimum effective daily dose of 1.40 DDDs of NAC necessary for achieving a meaningful reduction in HCC risk among HBV patients. The underlying mechanisms driving this protective effect warrant further investigation through comprehensive experimental studies, potentially illuminating new pathways in HBV-associated carcinogenesis and its prevention.

This study boasts several notable strengths, including a large sample size that significantly enhances the statistical power and generalizability of our findings. The inclusion of a substantial validation cohort further underpins the robustness and reliability of our results. The deployment of PSM ensured a homogenous distribution of covariates across study groups, effectively mitigating potential confounding influences and bolstering the legitimacy of our comparative analyses. Furthermore, the longitudinal verification of medication records in our dataset lends credence to our conclusions, facilitating an in-depth examination of the prolonged effects of medication use over time.

However, our study is not without its limitations. A primary concern lies in the demographic composition of our cohort, which consisted solely of Asian HBV carriers. Consequently, the extrapolation of our findings to other ethnicities necessitates further validation through additional studies. Additionally, under Taiwan's National Health Insurance system, NAC is predominantly prescribed for its mucolytic properties. This specificity in prescription purpose renders it challenging to discern the exact motivations behind NAC prescriptions in our cohort. While all known indications for NAC use were matched and accounted for in our analysis (as shown in **Table 1**), the direct causal relationship between NAC use and the observed reduction in HCC incidence remains ambiguous, potentially representing a bystander effect rather than a direct therapeutic consequence. Another notable limitation is the lack of data on patient adherence to NAC prescriptions. This gap introduces a potential bias, as non-adherence could confound the observed association between NAC use and HCC risk reduction. However, it is important to note that poor adherence would likely lead to an underestimation of NAC's effect on reducing HCC risk, rather than invalidating our conclusions. In summation, while our study provides important insights into the potential protective role of NAC against HCC in HBV carriers, these limitations necessitate a cautious interpretation of our results. The findings underscore the need for further research, particularly randomized controlled trials, to more definitively establish the efficacy of NAC in this context.

Conclusion

Our study presents compelling evidence for the potential protective role of NAC against HCC in HBV carriers. Leveraging a large-scale, real-world dataset, our findings reveal a significant association between NAC use and a reduced incidence of HCC in this high-risk group. The dose-response relationship observed underscores the importance of adequate NAC dosing in achieving optimal protective effects.

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Disclosure of conflict of interest

None.

Abbreviations

HCC, Hepatocellular Carcinoma; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; GSH, Glutathione; NAC, N-Acetylcysteine; ROS, Reactive Oxygen Species; NHI, National Health Insurance; NHIRD, National Health Insurance Research Database; cDDD, Cumulative Defined Daily Doses; DDD, Defined Daily Dose; Q, Quartile; IRB, Institutional Review Board; PSM, Propensity Score Matching; ICD, International Classification of Diseases; ICD-9-CM, International Classification of Diseases, Ninth Revision, Clinical Modification; ICD-10-CM, International Classification of Diseases, Tenth Revision, Clinical Modification; CCI, Charlson Comorbidity Index; IR, Incidence Rate; IRR, Incidence Rate Ratio; CI, Confidence Interval; HR, Hazard Ratio; aHR, Adjusted Hazard Ratio; DNA, Deoxyribonucleic Acid; RCT, Randomized Controlled Trial; HBx, Hepatitis B Virus X protein; NTD, New Taiwan Dollar; SD, Standard Deviation; IQR, Interquartile Range; NASH, Non-Alcoholic Steatohepatitis; COPD, Chronic Obstructive Pulmonary Disease; CKD, Chronic Kidney Disease.

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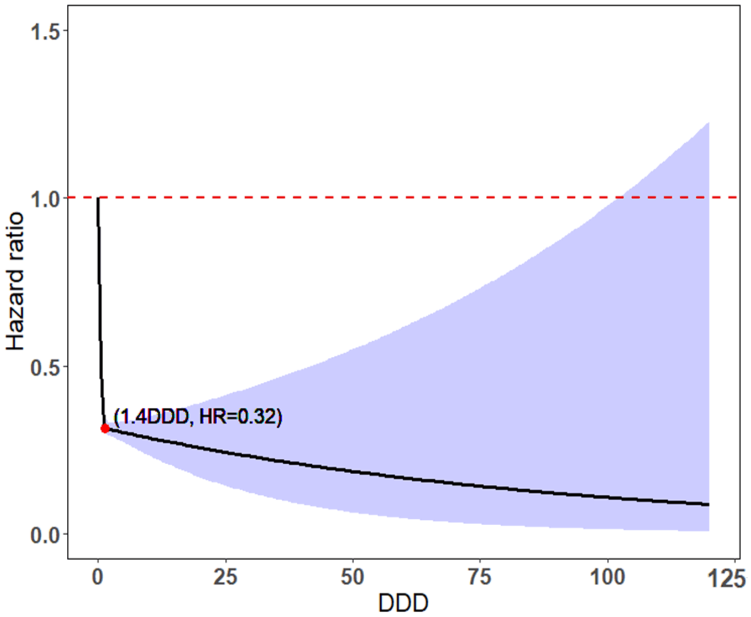

Supplementary Figure 1. Relationship between the intensity of N-acetylcysteine use (DDD) and the hazard ratio for hepatocellular carcinoma in chronic hepatitis B carriers.