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Original Article

Effects of N-acetylcysteine on hepatocellular carcinoma in chronic hepatitis C

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Abstract: Hepatitis C virus (HCV) infection significantly contributes to global hepatocellular carcinoma (HCC) incidence. N-Acetylcysteine (NAC), known for its antioxidant properties, is a potential therapeutic agent. However, evidence on its efficacy in reducing HCC risk among HCV patients is limited. A retrospective cohort analysis using Taiwan's National Health Insurance Research Database (2008-2018) included ≥18-year-old HCV patients. NAC usage (≥28 cumulative defined daily doses [cDDDs]) was assessed for its association with HCC risk using Cox regression models and propensity score matching. The study comprised 269,647 HCV patients, with detailed NAC dosage characterization and hazard ratios (HRs) for HCC risk. Post-matching, NAC usage emerged as the significant predictor of reduced HCC risk (adjusted HR: 0.39, 95% CI: 0.37-0.41, P<0.0001). Dose-response analysis showed reduced HCC risk with increasing cDDDs of NAC (P<0.0001). Higher daily NAC dosage (≥1 DDD) was associated with significantly lower HCC risk (adjusted HR: 0.33, 95% CI: 0.31-0.36, P<0.0001). The study provides compelling evidence for NAC's potential in reducing HCC risk among HCV patients. Insights into dose-dependent effects and optimal daily intensity thresholds offer valuable directions for future therapeutic strategies and clinical trials targeting HCC burden in HCV-infected individuals.

Keywords: N-Acetylcysteine, chronic hepatitis C, hepatocellular carcinoma, risk reduction, dose-response relationship

Introduction

Hepatitis C virus (HCV) is a bloodborne RNA virus, with a global prevalence estimated at 2-3%, affecting approximately 130-170 million individuals [1]. Despite the relatively low-to-intermediate prevalence of HCV in many Asian countries, this geographic region accounts for half of the global population infected with HCV. Following acute HCV infection, approximately 75% of patients progress to chronic infection, with around 20% of chronic hepatitis C patients developing cirrhosis within 10 years [2]. Moreover, between 1.9% and 6.7% of these

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individuals are estimated to develop hepatocellular carcinoma (HCC) over twenty years of HCV infection [3]. Since 2014, combination therapies involving various direct-acting antivirals (DAAs) have markedly improved sustained virologic response (SVR) rates for HCV treatment, increasing from 50% with previous interferon alfa treatment to 95% with these therapies [4]. Despite the significant reduction in HCC incidence associated with DAA-induced SVR [5], patients with cirrhosis prior to HCV SVR treatment continue to face a high risk of HCC (>2% per year) for up to ten years, even with a decrease in their fibrosis-4 score [6]. Additionally, since antiviral therapy for HCV was not widely reimbursed by Taiwan's National Health Insurance scheme at an early stage, we were able to assess the protective effects of NAC on the reduction of HCC risk in HCV patients. Thus, HCV infection remains a significant contributor to the burden of HCC incidence.

N-Acetylcysteine (NAC), known for its direct antioxidant properties and ability to boost intracellular glutathione (GSH) levels, particularly in hepatic cells, is widely utilized as a mucolytic agent for conditions like chronic bronchitis, pneumonia, and cystic fibrosis [7]. Additionally, it serves as an antidote for acetaminophen (paracetamol) overdose. Recent studies have explored NAC's antioxidant effects in various medical conditions, including contrast-induced nephropathy, cardiovascular disease, diabetes, neuropsychiatric disorders, and anti-carcinogenesis [8]. However, there is currently insufficient evidence to conclusively demonstrate NAC's efficacy in reducing the risk of HCC in HCV patients.

Hence, we conducted a real-world database study to assess the potential protective effects of NAC in reducing the incidence of HCC among patients with HCV infection. Furthermore, our objective was to investigate any potential dose-dependent relationship between NAC administration and the reduction in HCC risk in HCV patients. The primary aim of this study was to determine the effectiveness of NAC in preventing the progression of HCC in individuals with chronic hepatitis C.

Methods

Study cohort

In this retrospective cohort analysis covering the years 2008 to 2018, we leveraged Taiwan's National Health Insurance Research Database (NHIRD) to investigate individuals carrying HCV [9]. The NHIRD provides comprehensive data covering over 99% of the Taiwanese populace, encompassing encrypted records of diagnoses, medical interventions, and medication prescriptions [9]. Furthermore, through linkage with Taiwan's death registry, we were able to ascertain the mortality status and causes of death among the participants, offering a comprehensive outlook for our study [9].

In our examination of patients with HCV, we employed data from the NHIRD, focusing on individuals aged 18 years and older, with complete age documentation. NAC administration was defined as the receipt of 28 or more cumulative defined daily doses (cDDDs) subsequent to HCV diagnosis. The study period commenced at the initiation of NAC treatment and continued until the occurrence of HCC diagnosis, patient demise, or December 31, 202. Patients were stratified into two cohorts: those who received at least 28 cDDDs of NAC constituted the case group, while individuals without any NAC prescriptions comprised the control group. The follow-up duration encompassed one year from the commencement of NAC treatment or from the date of cohort entry. The primary aim of this investigation was to delineate the relationship between NAC utilization and the risk of developing HCC in patients with HCV.

In our examination of patients with HCV, stringent exclusion criteria were applied to maintain data integrity. Individuals were excluded from the analysis if they: (1) received an HCC diagnosis within one year of the index date; (2) lacked complete sex or age data, or were under the age of 18; (3) were followed for less than a year; (4) had a prior history of any cancer diagnosis before cohort entry; or (5) initiated NAC therapy before their HCV diagnosis. These exclusions were crucial in ensuring the accuracy and reliability of our study's findings, particularly concerning the impact of NAC utilization on the risk of HCC in HCV-infected individuals.

The study protocols obtained ethical clearance from the Institutional Review Board (IRB) of the Tzu-Chi Medical Foundation, as evidenced by approval number IRB109-015-B.

Exposure to N-Acetylcysteine

NAC usage was defined as the administration of at least 28 cDDDs. While primarily utilized as

a mucolytic agent in respiratory conditions like chronic bronchitis to enhance mucus clearance and respiratory function [10], NAC's applications are diverse. To account for potential fluctuations in NAC consumption over the study period, we treated NAC usage as a time-varying covariate in our Cox regression analysis.

We calculated the cumulative dose of NAC by dividing the total prescribed amount by the days of supply. Adhering to the World Health Organization's standard, we quantified NAC dosage using the defined daily dose (DDD) metric, which represents the average maintenance dosage per day for an adult's primary indication. To assess the impact of NAC's daily dose intensity on HCC risk, we categorized usage into two groups: ≥1 DDD indicating significant daily use, and <1 DDD. The cDDDs were aggregated to ensure a minimum of 28 cDDDs, distinguishing NAC use (≥28 cDDDs) from nonuse (0 cDDD). Furthermore, the study cohort was divided into quartiles based on cDDD stratification to enable detailed analysis.

Propensity score matching and covariate analysis

To adjust for potential confounding variables, we included a comprehensive set of covariates in our analysis. Participants were categorized into four age groups: 18-44, 45-54, 55-64, and ≥65 years. The index date for NAC users was defined as the start of NAC treatment, marked by a minimum cumulative intake of 28 cDDDs. For matched non-NAC users, the index date corresponded to the date of equivalent variable assessment. Comorbidities diagnosed within a year of the index date were classified using International Classification of Diseases (ICD) codes, derived from primary inpatient diagnoses or at least two outpatient visits within the year. We utilized both ICD-9-CM and ICD-10-CM for accurate coding and ensured no overlap between Charlson Comorbidity Index (CCI) scores and individual comorbidities. A time-varying Cox proportional hazards model was employed to evaluate the relationship between NAC use and HCC development, adjusting for potential confounders. Propensity score matching (PSM) was utilized for a robust comparison of HCC risk between NAC users and nonusers, with matching parameters including age, sex, income, urbanization level, CCI scores, existing comorbidities, and specific medications. Continuous variables were presented as means ± standard deviations or medians with interquartile ranges based on their distribution characteristics. The greedy algorithm in PSM with a caliper width of 0.1 was applied to establish a 1:1 match [11], systematically pairing patients and controls based on critical covariates identified by our team to control potential confounders.

Main outcome measures

The primary outcome of the study was the incidence of HCC, confirmed through certification records from the Catastrophic Illness Patient Registry [12].

Statistical methods and analysis

Patient characteristics, detailed in **Table 1**, were incorporated as covariates, with age groups categorized into decade-long intervals. Baseline characteristics between NAC users and nonusers were compared using chi-squared tests for categorical variables, t-tests for continuous variables, and Wilcoxon rank-sum tests for medians. The date of cohort entry served as the baseline for analysis.

To assess the association between NAC use and HCC risk, we calculated incidence rates (IRs) and incidence rate ratios (IRRs). Adjusted hazard ratios (aHRs) with 95% confidence intervals (CIs) were estimated using Cox regression models, incorporating variables such as age, sex, income, urbanization level, CCI scores, prevalent comorbidities, and medication use, as specified in **Table 1**. Time-varying Cox regression was further employed to evaluate the effects of varying cDDDs of NAC and its daily intensity (≥1 DDD or <1 DDD) on HCC risk in HCV patients.

A time-dependent Cox hazard model was utilized to compare HCC risk in NAC users versus non-users, adjusting for the aforementioned confounders. NAC prescription data, updated every three months, determined the user status and was treated as a time-varying variable. Additionally, the Fine and Gray model was applied to account for the risk of ischemic stroke as a competing event. Cumulative incidence of HCC was estimated using the Kaplan-

Table 1. Characteristics comparison of chronic hepatitis C patients pre- and post-propensity score matching, with versus without N-acetylcysteine

	Before PSM					After PSM				
	Never-NAC use		NAC Use		ASMD	Never-NAC use		NAC Use		ASMD
	N=229,315		N=40,332			N=40,015		N=40,015		
	N	%	N	%		N	%	N	%	
Age, years-old (mean ± SD)	52.98 ± 15.17		61.85 ± 14.35			61.42 ± 13.94		61.75 ± 14.34		
Age, median (IQR), y	53.00 (42.00, 64.00)		63.00 (52.00, 73.00)			63.00 (52.00, 72.00)		63.00 (52.00, 73.00)		
Age group, years					0.3708					0.0240
18-44	67,380	29.38%	5,067	12.56%		4,748	11.87%	5,067	12.66%	
45-54	54,471	23.75%	7,043	17.46%		7,064	17.65%	7,038	17.59%	
55-64	50,238	21.91%	9,099	22.56%		9,144	22.85%	9,076	22.68%	
≥65	57,226	24.96%	19,123	47.41%		19,059	47.63%	18,834	47.07%	
Sex					0.0136					0.0224
Female	117,355	51.18%	20,368	50.50%		20,677	51.67%	20,227	50.55%	
Male	111,960	48.82%	19,964	49.50%		19,338	48.33%	19,788	49.45%	
Income (NTD)					0.2581					0.0130
Low income	51,958	22.66%	11,067	27.44%		10,942	27.34%	10,966	27.40%	
≤20,000	120,887	52.72%	23,043	57.13%		23,019	57.53%	22,835	57.07%	
20,001-30,000	26,840	11.70%	3,305	8.19%		3,225	8.06%	3,298	8.24%	
30,001-45,000	18,281	7.97%	1,943	4.82%		1,864	4.66%	1,942	4.85%	
>45,000	11,349	4.95%	974	2.41%		965	2.41%	974	2.43%	
Urbanization					0.1475					0.0050
Rural	86,272	37.62%	18,094	44.86%		18,009	45.01%	17,912	44.76%	
Urban	143,043	62.38%	22,238	55.14%		22,006	54.99%	22,103	55.24%	
CCI Scores										
Mean (SD)	1.91 ± 1.36		2.51 ± 1.65			2.36 ± 1.53		2.51 ± 1.65		
Median (Q1-Q3)	2.00 (1.00, 2.00)		2.00 (2.00, 3.00)			2.00 (2.00, 3.00)		2.00 (2.00, 3.00)		
CCI Scores					0.2216					0.0041
0	48,790	21.28%	5,235	12.98%		5,290	13.22%	5,234	13.08%	
≥1	180,525	78.72%	35,097	87.02%		34,725	86.78%	34,781	86.92%	
CCI										
Congestive Heart Failure	8,215	3.58%	3,776	9.36%	0.2366	3,346	8.36%	3,727	9.31%	0.0335
Dementia	1,931	0.84%	1,458	3.61%	0.1886	852	2.13%	1,407	3.52%	0.0840
Chronic Pulmonary Disease	25,610	11.17%	13,439	33.32%	0.5525	13,068	32.66%	13,184	32.95%	0.0007
Rheumatic Disease	6,469	2.82%	1,763	4.37%	0.0833	1,374	3.43%	1,751	4.38%	0.0491

Liver Disease	164,430	71.70%	28,999	71.90%	0.0044	30,021	75.02%	28,788	71.94%	0.0698
Diabetes with complications	9,348	4.08%	2,952	7.32%	0.1401	2,975	7.43%	2,933	7.33%	0.0038
Hemiplegia and Paraplegia	2,233	0.97%	952	2.36%	0.1088	662	1.65%	929	2.32%	0.0480
Renal Disease	11,915	5.20%	3,998	9.91%	0.1789	3,739	9.34%	3,968	9.92%	0.0197
AIDS	2,600	1.13%	144	0.36%	0.0896	200	0.50%	144	0.36%	0.0214
Cancer	9,523	4.15%	2,920	7.24%	0.1336	2,271	5.68%	2,889	7.22%	0.0627
Coexisting comorbidities										
Diabetes	44,130	19.24%	11,457	28.41%	0.2165	11,111	27.77%	11,364	28.40%	0.0140
Hypertension	75,554	32.95%	21,331	52.89%	0.4113	20,994	52.47%	21,072	52.66%	0.0038
Hyperlipidemia	40,535	17.68%	9,642	23.91%	0.1540	9,348	23.36%	9,574	23.93%	0.0134
Non-alcoholic steatohepatitis (NASH)	18,402	8.02%	4,252	10.54%	0.0869	3,972	9.93%	4,225	10.56%	0.0208
Alcohol-related liver diseases	10,148	4.43%	1,934	4.80%	0.0176	1,826	4.56%	1,924	4.81%	0.0118
Liver cirrhosis	9,226	4.02%	3,327	8.25%	0.1770	3,205	8.01%	3,300	8.25%	0.0088
Cholelithiasis	15,371	6.70%	3,922	9.72%	0.1102	3,595	8.98%	3,896	9.74%	0.0261
COPD	30,719	13.40%	15,896	39.41%	0.6175	15,313	38.27%	15,579	38.93%	0.0136
Pneumonia	12,387	5.40%	8,008	19.86%	0.4460	6,721	16.80%	7,693	19.23%	0.0633
Bronchitis	83,621	36.47%	21,397	53.05%	0.3382	20,917	52.27%	21,157	52.87%	0.0120
Pulmonary cystic fibrosis	2	0.00%	0	0.00%	0.9999	0	0.00%	0	0.00%	0.0000
Myocardial infarction	1,925	0.84%	944	2.34%	0.1201	777	1.94%	929	2.32%	0.0263
Congestive heart failure	9,043	3.94%	4,780	11.85%	0.2965	4,290	10.72%	4,721	11.80%	0.0342
Cerebrovascular disease	19,327	8.43%	8,257	20.47%	0.3476	7,733	19.33%	8,082	20.20%	0.0218
Obesity	1,860	0.81%	388	0.96%	0.0160	332	0.83%	385	0.96%	0.0138
Ascites	2,639	1.15%	555	1.38%	0.0206	501	1.25%	549	1.37%	0.0106
Hepatic coma	1,357	0.59%	314	0.78%	0.0230	285	0.71%	313	0.78%	0.0081
Medication use										
Anti-HCV treatment	35,174	15.34%	5,099	12.64%	0.0779	5,187	12.96%	5,091	12.72%	0.0072
Statins	42,402	18.49%	10,938	27.12%	0.2068	10,910	27.26%	10,872	27.17%	0.0020
Metformin	42,882	18.70%	10,499	26.03%	0.1766	10,485	26.20%	10,441	26.09%	0.0025
Aspirin	63,274	27.59%	20,229	50.16%	0.4759	20,573	51.41%	19,928	49.80%	0.0322
NAC, cDDD										
Mean (sd)	0.00		188.11 ± 336.42			0.00		186.36 ± 333.50		
Median (q1, q3)	0.00		73.00 (42.02, 171.61)			0.00		72.80 (42.02, 169.40)		
NAC, cDDD										
Never use	229,315	100.00%	0	0.00%		40,015	100.00%	0	0.00%	
Q1	0	0.00%	9,884	24.51%		0	0.00%	9,850	24.62%	

Q2	0	0.00%	10,278	25.48%		0	0.00%	10,225	25.55%	
Q3	0	0.00%	10,100	25.04%		0	0.00%	10,041	25.09%	
Q4	0	0.00%	10,070	24.97%		0	0.00%	9,899	24.74%	
DDD										
Never use	229,315	100.00%	0	0.00%		40,015	100.00%	0	0.00%	
<1	0	0.00%	21,981	54.50%		0	0.00%	21,803	54.49%	
≥1	0	0.00%	18,351	45.50%		0	0.00%	18,212	45.51%	
Mean (SD) follow-up time, year	6.69 ± 3.84		6.83 ± 3.75			6.00 ± 3.65		6.85 ± 3.75		
Median (IQR) follow-up time, year	6.37 (3.35, 9.72)		6.54 (3.56, 9.77)			5.48 (2.87, 8.67)		6.57 (3.58, 9.79)		
Primary outcome					*P*-value					*P*-value
HCC	23,206	10.12%	2,245	5.57%	<0.0001	4,694	11.73%	2,236	5.59%	<0.0001

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

Table 2. Hepatocellular carcinoma risk in matched chronic hepatitis C patients: N-acetylcysteine usage analysis by intensity and cumulative dose

	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	0.39 (0.37, 0.41)	<0.0001	0.40 (0.38, 0.42)	<0.0001
cDDD of NAC (ref. Never-NAC use)	1.00	-	1.00	-	1.00	-
Q1	0.48 (0.44, 0.52)	<0.0001	0.51 (0.47, 0.56)	<0.0001	0.53 (0.48, 0.57)	<0.0001
Q2	0.46 (0.42, 0.5)	<0.0001	0.47 (0.43, 0.51)	<0.0001	0.48 (0.44, 0.52)	<0.0001
Q3	0.40 (0.36, 0.43)	<0.0001	0.37 (0.34, 0.40)	<0.0001	0.37 (0.34, 0.41)	<0.0001
Q4	0.31 (0.28, 0.35)	<0.0001	0.24 (0.22, 0.27)	<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	0.47 (0.44, 0.5)	<0.0001	0.44 (0.41, 0.46)	<0.0001	0.44 (0.41, 0.47)	<0.0001
≥1	0.34 (0.32, 0.37)	<0.0001	0.33 (0.31, 0.36)	<0.0001	0.35 (0.32, 0.37)	<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; cDDD, cumulative defined daily dose; DDD, defined daily dose; 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.

Meier method and compared across groups using the log-rank test.

All statistical analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC), with statistical significance set at a two-sided *P*-value below 0.05.

Results

Demographic and clinical profiles of chronic hepatitis C patients

Our study analyzed data from 269,647 patients with HCV enrolled between 2008 and 2018, as delineated in **Table 1**. Before implementing PSM, the NAC user cohort comprised predominantly older individuals with lower income levels, rural residency, elevated CCI scores, and a higher prevalence of comorbidities including diabetes, hypertension, hyperlipidemia, liver cirrhosis, cholelithiasis, chronic obstructive pulmonary disease, pneumonia, bronchitis, pulmonary cystic fibrosis, myocardial infarction, congestive heart failure, and cerebrovascular disease.

To ensure comparability, we implemented 1:1 PSM, resulting in two balanced cohorts of 40,015 patients each. Post-PSM, the age distributions in both cohorts were comparable, as demonstrated in **Table 1**. Following PSM, key variables including age, sex, income, urbanization, CCI scores, prevalent comorbidities, and medication usage exhibited no significant statistical differences between NAC users and non-users. Subsequent to PSM, the observed incidence of HCC was 5.59% in the NAC group compared to 11.73% in the group that never used NAC (P<0.0001).

HCC risk comparison: HCV patients with and without NAC use

Following PSM, none of the covariates outlined in **Table 1** exhibited a notable association with HCC risk. Notably, NAC use emerged as the sole significant independent predictor. The aHR for HCC in the NAC-using cohort, relative to those not using NAC, was 0.39 (95% CI: 0.37-0.41, P<0.0001), as delineated in **Table 2**. Furthermore, when accounting for the competing risk of mortality, the aHR for HCC in NAC users versus non-users was 0.40 (95% CI: 0.38-0.42).

NAC dose-response and HCC risk among HCV patients

In our exploration of the dose-response correlation between NAC utilization and HCC risk among HCV patients, cDDDs were stratified into four quartiles (Q1, Q2, Q3, Q4), as outlined in **Table 2**. The aHRs for HCC in these quartiles

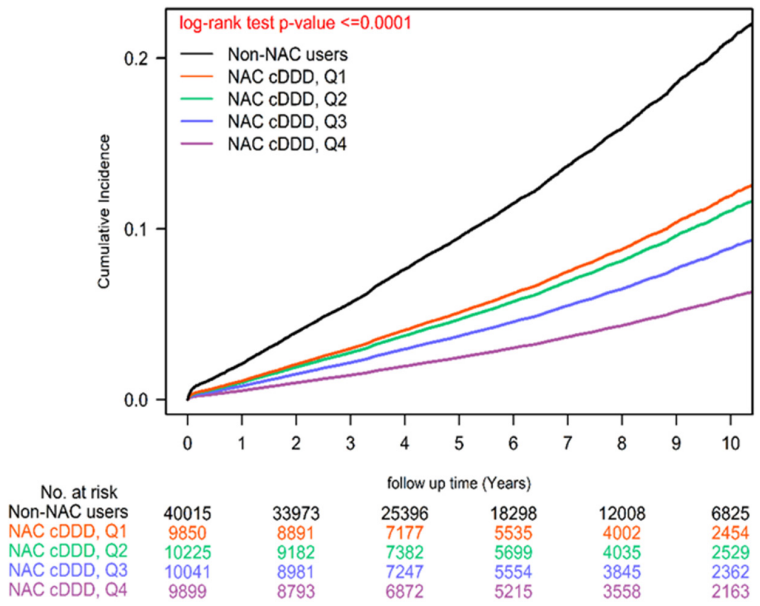

Figure 1. Kaplan-Meier curves for cumulative HCC incidence in chronic hepatitis C cohort, stratified by NAC cDDD categories.

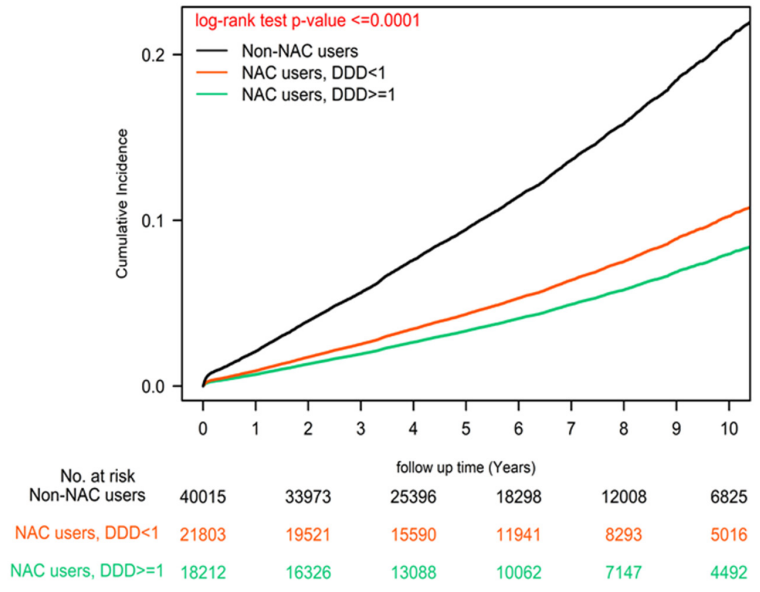

Figure 2. Kaplan-Meier plots of HCC cumulative incidence in chronic hepatitis C patients, stratified by NAC use at varying daily intensity levels (DDD).

relative to non-NAC users were as follows: Q1, 0.51 (95% CI, 0.47-0.56); Q2, 0.47 (95% CI, 0.43-0.51); Q3, 0.37 (95% CI, 0.34-0.40); and Q4, 0.24 (95% CI, 0.22-0.27). A notable dose-response trend was observed (P<0.0001), signifying a reduction in HCC risk with escalating NAC usage. This trend persisted even after adjusting for mortality as a competing risk. Furthermore, Kaplan-Meier analysis unveiled a substantially lower cumulative incidence of HCC in the higher NAC quartiles (Q4 to Q1) compared to non-NAC users (**Figure 1**, P< 0.0001).

Effect of NAC daily dose intensity on HCV-related HCC risk

In our examination of the impact of daily NAC dosage intensity on HCC risk among HCV patients, we classified the DDD into two categories: DDD<1 and DDD≥1, as depicted in **Table 2**. The aHRs for HCC in these groups, compared to non-NAC users, were 0.44 (95% CI, 0.41-0.46) for DDD<1 and 0.33 (95% CI, 0.31-0.36) for DDD≥1. A significant association (P<0.0001) indicated that higher daily NAC doses correlated with reduced HCC risk in HCV patients. This correlation remained robust after adjusting for mortality as a competing risk. Kaplan-Meier analysis further illustrated a markedly lower cumulative incidence of HCC in patients with DDD≥1 NAC usage, followed by the DDD<1 group and non-NAC users (**Figure 2**, P< 0.0001).

Supplementary Figure 1 portrays the relationship between the daily intensity of NAC use, quantified in DDD, and the hazard ratio for HCC in chronic hepatitis C patients. The findings suggest that a daily dosage of 1.50 DDD may represent the threshold for reducing HCC risk. Beyond this threshold (NAC>1.50 DDD), the decline in HCC risk appears to plateau, although a continual, albeit gradual, risk reduction is observed with increasing NAC daily intensity dosages.

Table 3. Incidence and hazard ratios for HCC in matched chronic hepatitis C cohort: N-acetylcysteine analysis by use intensity and cumulative dose

	Events	Person-years	IR (per 10,000 person-year)	IRR	95% CI for IRR	*P*
NAC use						
Never-NAC use	4,694	240,210.7	195.41	Ref.		
NAC use	2,236	274,066.7	81.59	0.42	(0.40, 0.44)	<0.0001
NAC use (cDDD)						
Never-NAC use	4,694	240,210.7	195.41	Ref.		
NAC user dose, Q1	649	68,709.7	94.46	0.48	(0.45, 0.52)	<0.0001
NAC user dose, Q2	646	70,976.2	91.02	0.47	(0.43, 0.51)	<0.0001
NAC user dose, Q3	538	68,793.2	78.21	0.40	(0.37, 0.44)	<0.0001
NAC user dose, Q4	403	65,587.7	61.44	0.31	(0.28, 0.35)	<0.0001
NAC use (daily density, DDD)						
Never-NAC use	4,694	240,210.7	195.41	Ref.		
<1	1,378	148,059.3	93.07	0.48	(0.45, 0.51)	<0.0001
≥1	858	126,007.4	68.09	0.35	(0.32, 0.37)	<0.0001

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

Comparative analysis of HCC incidence in NAC users versus non-users

Table 3 illustrates the association between NAC utilization and the incidence of HCC within our HCV patient cohort. Notably, the incidence rate of HCC per 10,000 person-years was markedly lower in NAC users (81.95) than in non-users (195.41). The incidence rate ratio (IRR) for HCC among NAC users, with a 95% CI, stood at 0.42 (0.40-0.44) compared to non-users.

Further analysis unveiled a dose-response relationship, indicating that increased NAC usage corresponded to a diminished risk of HCC. The IRRs for HCC across NAC usage quartiles (Q1, Q2, Q3, Q4) were 0.48 (0.45-0.52), 0.47 (0.43-0.51), 0.40 (0.37-0.44), and 0.31 (0.28-0.35), respectively, relative to non-users. A similar trend was observed when evaluating the influence of daily dose density on HCC risk. For NAC usage at DDD<1 and DDD≥1, the IRRs were 0.48 (0.45-0.51) and 0.35 (0.32-0.37), respectively, compared to those who had never used NAC.

Discussion

This study revealed a significant reduction in the risk of developing HCC among chronic HCV patients, with NAC usage emerging as the sole significant independent predictor. Moreover, a notable decrease in the cumulative incidence of HCC was observed in the higher NAC quartiles compared to non-users. Although the therapeutic efficacy of NAC in HCV treatment remains debatable, several studies have highlighted its potential benefits. For instance, de Oliveira CP et al. found that NAC combined with metformin over 12 months led to improved histological activity scores and reduced hepatic fibrosis in patients with non-alcoholic steatohepatitis [13]. A systematic review and meta-analysis by Amjad W et al. demonstrated a significant improvement in transplant-free survival with NAC treatment [14]. Additionally, Nabi T et al. observed a reduction in mortality, shorter hospital stays, and improved survival rates in non-acetaminophen-induced acute liver failure patients treated with NAC compared to controls [15].

Chronic hepatitis C is characterized by persistent liver injury marked by inflammation, necrosis, and fibrosis, which can progress to cirrhosis and ultimately HCC [16]. The pathogenesis of chronic hepatitis C involves an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms. HCV induces endoplasmic reticulum (ER) stress, leading to the release of calcium ions from the ER and mitochondria, resulting in oxidative stress (OS) characterized by increased ROS levels [17]. Additionally, HCV infection pro-

motes iron accumulation in hepatocytes, contributing to iron overload and the subsequent production of lipid hydroperoxides, further exacerbating ROS production [18]. Furthermore, HCV patients exhibit decreased serum levels of catalase and glutathione peroxidase, compromising their endogenous antioxidant defense mechanisms and exacerbating redox imbalance [19].

NAC serves as a precursor for cysteine in the synthesis of hepatic GSH, a vital intracellular antioxidant pivotal in safeguarding cells against OS. GSH acts as a primary defense mechanism against OS by neutralizing ROS such as hydrogen peroxide (H_2O_2), superoxide radicals (O2•-), and free radicals. Moreover, GSH indirectly sustains the active state of well-known antioxidants such as vitamins C and E [20]. Additionally, GSH plays a critical role in detoxifying reactive metabolites by forming conjugates with electrophilic compounds and xenobiotics [21]. Furthermore, GSH functions as a signaling molecule, intricately involved in regulating diverse cellular processes, including cell proliferation, apoptosis, and gene expression [22].

NAC contains a thiol group derived from cysteine, which can be oxidized by various radicals, including chelating transition metals such as Cu2+, Fe3+, and heavy metals like Cd2+, Hg2+, and Pb2+, through its thiol chain [23]. Moreover, NAC, functioning as a potent reducing agent, exhibits greater efficacy than cysteine and GSH in directly neutralizing reactive oxygen and nitrogen species (RONS) [24]. Additionally, NAC mitigates the activation of nuclear factor κ-light-chain enhancer of activated B cells (NF-κB), thereby inhibiting Kupffer cells' activation and the subsequent release of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), and IL-6 [25].

In summary, despite not being commonly utilized in HCV treatment protocols, NAC exhibits potential therapeutic effects, including: (i) elevation of hepatic GSH levels and antioxidant activity; (ii) inhibition of inflammatory cytokine release; (iii) chelation of transition and heavy metals; and (iv) regulation of cell cycle and apoptosis. Consequently, owing to its anti-inflammatory properties, NAC holds promise in impeding the progression of liver fibrosis and the onset of cirrhosis. Given the aforementioned potential mechanisms of NAC's protective effects in reducing HCC risk for HCV patients, further randomized controlled trials are needed to confirm these effects.

To date, comprehensive investigations into the IRRs, HRs, cDDDs, and daily intensity of NAC use in relation to HCC risk among HCV patients have been lacking (**Tables 2**, **3** and **Figure 1**). Our study represents a pioneering effort, providing detailed insights into NAC dosage and its association with HCC risk in individuals with chronic HCV infection. By delineating the dose-dependent effects of NAC on HCC risk and identifying optimal daily intensity thresholds (**Figure 2** and Supplementary Figure 1), we offer novel perspectives crucial for guiding future clinical trials and elucidating the underlying mechanisms of NAC in reducing HCC risk in the context of HCV infection. This groundbreaking exploration serves as a cornerstone for advancing the understanding and potential therapeutic applications of NAC in mitigating the burden of HCC in HCV patients.

The strength of this study lies in its comprehensive evaluation of NAC as a potential therapeutic agent for reducing the risk of HCC in patients with chronic HCV infection. Given the substantial global burden of HCV-related HCC, particularly in Asian populations, where half of the worldwide HCV-infected individuals reside, there is a pressing need for effective preventive strategies. While DAAs have significantly improved sustained virologic response rates, patients with pre-existing cirrhosis remain at elevated risk of HCC even after achieving viral clearance. Furthermore, due to the limited availability of antiviral therapy for HCV under Taiwan's National Health Insurance scheme during the early stages, we were afforded the opportunity to evaluate the protective effects of NAC in reducing the risk of HCC among HCV patients. NAC, renowned for its antioxidant properties and ability to augment intracellular glutathione levels, presents a promising avenue for mitigating HCC risk in this vulnerable population. By leveraging real-world data from Taiwan's National Health Insurance Research Database (NHIRD), this study provides robust evidence supporting the protective effects of NAC against HCC development in HCV-infected individuals. Through meticulous propensity score matching and comprehensive adjustment for confounding variables, the study eluci-

dates a significant dose-response relationship between NAC utilization and reduced HCC incidence. Furthermore, the findings underscore the potential of NAC to serve as an adjunctive therapy in the management of chronic hepatitis C, offering a novel approach to attenuating the progression of liver fibrosis and curbing the onset of cirrhosis. This study fills a critical gap in the literature by shedding light on the therapeutic potential of NAC in the context of HCV-related HCC, paving the way for future research endeavors aimed at optimizing treatment strategies and improving clinical outcomes in this high-risk patient population.

There were several limitations in this study. First, we could only estimate treatment durations of NAC by dividing the cumulative doses of individual medications by DDD, which may not accurately reflect actual usage patterns. Second, the NHIRD did not provide personal information such as body mass index, lifestyle factors, family history, laboratory results, and imaging data. Consequently, the severity of liver cirrhosis in each patient could not be assessed, potentially influencing our findings. Further research incorporating comprehensive clinical data is required to accurately evaluate the effectiveness of NAC usage for preventing the progression of HCC in chronic HCV patients.

Conclusion

Our study reveals a significant association between NAC use and reduced HCC risk in chronic hepatitis C patients. Through rigorous analysis of real-world data, we demonstrate a dose-dependent relationship, indicating higher NAC doses correlate with greater HCC risk reduction. These findings suggest the potential therapeutic role of NAC in managing HCV-related HCC, highlighting the need for further prospective studies to validate and elucidate its mechanisms.

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Disclosure of conflict of interest

None.

Abbreviations

HCV, hepatitis C; NAC, N-Acetylcysteine; HCC, hepatocellular carcinoma; GSH, hepatic glutathione; cDDDs, cumulative defined daily doses; PSM, propensity score matching; DAAs, direct-acting antivirals; SVR, sustained virologic response.

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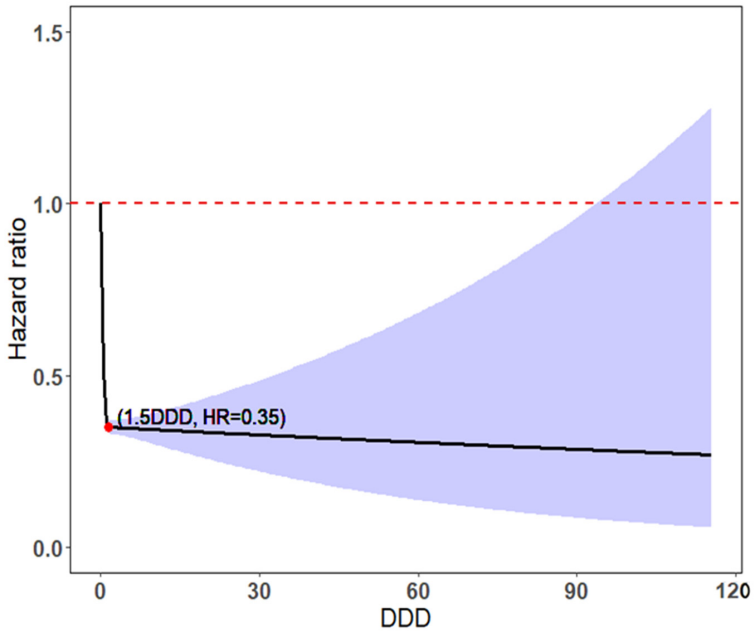

Supplementary Figure 1. Correlation of N-acetylcysteine use intensity (DDD) with hepatocellular carcinoma hazard ratio in chronic hepatitis C patients.