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Association between the non-high-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio (NHHR) and mortality in patients with metabolic dysfunction-associated steatotic liver disease (MASLD): data from the NHANES III (1988–1994)

Ying Zhang[1], Peng-Yu Luo[1], Yu-Na Tang[1], Jing Wang[1], Shuai Gao[1], Yu-Chen Fan[1] and Kai Wang[1,2*]

Abstract

Background The prognostic value of the non-high-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio (NHHR) in patients with metabolic dysfunction-associated steatotic liver disease (MASLD) remains unclear. This study aimed to evaluate the associations between the NHHR and all-cause and cause-specific mortality in patients with MASLD.

Methods Data for this study were obtained from the National Health and Nutrition Examination Survey (NHANES III and the National Death Index (NDI). The NHHR was calculated according to the formula. The results of mortality associated with the NDI were recorded as of December 31, 2019. We used a multivariate Cox proportional hazard model and restricted cubic spline (RCS) regression to assess the associations between the NHHR and all-cause and cause-specific mortality. In addition, subgroup analyses were performed to explore the relationships between the NHHR and all-cause and cause-specific mortality.

Results This study included 3155 patients with a definite diagnosis of MASLD. A total of 1,381 (43.8%) patients with MASLD died, and 1,774 (56.2%) survived. Multivariate Cox proportional hazards model analysis showed that NHHR was not significantly associated with all-cause mortality in MASLD patients. The RCS curve showed a significant nonlinear trend between the NHHR and all-cause mortality in patients with MASLD. Subgroup analysis revealed that the NHHR was better suited to predict cardiovascular mortality in patients without advanced fibrosis.

*Correspondence:
Kai Wang
wangdoc876@126.com; wangdoc2010@163.com

Full list of author information is available at the end of the article

Conclusions Our study revealed the clinical value of the NHHR in the prediction of mortality in the MASLD population. The NHHR can be used as a biomarker for follow-up in people without advanced fibrosis.

Keywords MASLD, NHHR, Mortality, NHANES III

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic diseases, with an estimated prevalence of 25–30% in adults worldwide and many undiagnosed cases due to geographic factors or medical conditions [1]. In addition, NAFLD is associated with the development of metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), chronic kidney disease (CKD), obstructive sleep apnoea (OSA), liver decompensation, and hepatocellular carcinoma (HCC) [2]. Moreover, the latest data from the United Network for Organ Sharing (UNOS) indicate that NAFLD is the second most common and fastest growing indication for liver transplantation [3]. Therefore, NAFLD not only seriously impairs the quality of life of patients but also results in a severe economic burden [4]. The term "nonalcoholic" has long been considered an inaccurate term for capturing the cause of NAFLD, and some patients with NAFLD are excluded from treatment because of unreasonable diagnostic criteria. Therefore, in 2023, the multisociety statement recommended that NAFLD be renamed metabolic dysfunction-associated steatotic liver disease (MASLD) after discussion [5].

Patients with MASLD are twice as likely to develop dyslipidaemia as those without MASLD, and the subcomponents of lipids in patients with MASLD make them more likely to develop atherosclerosis [6, 7]. As MASLD progresses to cirrhosis, patients remain at high risk for atherosclerosis, although their blood lipids and lipoproteins may normalize owing to reduced liver synthesis [2]. The ratio of non-high-density lipoprotein cholesterol (non-HDL-C) to high-density lipoprotein cholesterol (HDL-C) (NHHR) is a new index for evaluating atherosclerosis. The NHHR has superior predictive and diagnostic efficacy in assessing the risk of atherosclerosis, chronic kidney disease, and T2DM [8–10]. In addition, recent studies have reported the association and predictive value of the NHHR in patients with various diseases, such as depression, periodontitis, and kidney stones [11–13]. Although the relationship between the NHHR and NAFLD has been reported in recent studies [14], the relationship between the NHHR and NAFLD may not be completely consistent with that between the NHHR and MASLD because the diagnostic criteria for NAFLD and MASLD differ. Studies comparing clinical features and mortality between the two diseases have shown significant differences [15].

The main objective of this research was to assess the relationship between the NHHR and all-cause/cause-specific mortality using data from the National Health and Nutrition Examination Survey (NHANES) and the National Death Index (NDI). This was a preliminary study on the relationship between the NHHR and the clinical outcomes of adult patients with MASLD, which may provide a theoretical basis for the clinical management of adult patients with MASLD.

Methods

The study used data from the National Death Index (NDI) and the National Health and Nutrition Examination Survey (NHANES III) databases for the period 1988–1994. The NHANES database website has a detailed description of the database. (https://www.cdc.gov/nchs/nhanes/). Similarly, the NDI database website has a detailed description of the database (https://www.cdc.gov/nchs/ndi/index.htm). A total of 20 050 patients were included in this study. After excluding 16 895 patients who were pregnant, did not meet the diagnostic criteria and had incomplete information, 3155 patients aged over 20 years were ultimately included. The use of NHANES data was formally approved by the National Center for Health Statistics Research Ethics Review Committee, and all participants signed informed consent forms.

Definitions of MASLD

People with hepatic steatosis (including mild, moderate, and severe steatosis) were diagnosed with steatotic liver disease (SLD). Patients with MASLD who were diagnosed with SLD and who met any of the following cardiometabolic criteria for adults were included after patients with viral hepatitis, autoimmune liver disease, hereditary liver disease, drug-induced liver disease, alcohol-related liver disease, or an alcohol intake ≥ 30 g/day in men and ≥ 20 g/day in women were excluded. The cardiometabolic criteria for adults were as follows [5]:

(1) A body mass index (BMI) ≥ 25 kg/m^2 or a waist circumference (WC) ≥ 94 cm for males and ≥ 80 cm for females;
(2) An Fasting Blood Glucose (FBG) level ≥ 100 mg/dL, a 2-h postload glucose level ≥ 140 mg/dL, a haemoglobin A1c level ≥ 5.7%, DM or the receipt of hypoglycaemic therapy for DM;
(3) A blood pressure ≥ 130/85 mmHg or the receipt of antihypertensive drug treatment;
(4) A fasting plasma triglyceride level ≥ 150 mg/dL or the receipt of lipid-lowering treatment;

(5) A plasma HDL-cholesterol level < 40 mg/dL for males and < 50 mg/dL for females or the receipt of lipid-lowering treatment.

Therefore, our inclusion and exclusion criteria are as follows: Inclusion criteria, (1) consistent with the diagnosis of MASLD; (2) age>20y; (3) complete information. Exclusion criteria, (1) viral hepatitis, autoimmune liver disease, hereditary liver disease, drug or drug-induced liver disease, alcohol-related liver disease, or alcohol intake ≥ 30 g/ day in men and ≥ 20 g/ day in women; (2) pregnant; (3) incomplete information.

Definitions of NHHR

The NHHR was calculated as follows [13]:

$$NHHR = \frac{Total\ cholesterol(TC) - High-density\ lipoprotein\ cholesterol(HDL-C)}{HDL-C}$$

Assessment of covariates

The covariates included in this study were sex (male, female), age, race (non-Hispanic white, non-Hispanic black, Mexican-American, other races), education level (< high school, high school, college), marital status, sedentary lifestyle status, smoking status, and waist circumference. We also recorded whether the participants had diabetes or high blood pressure and whether they were taking insulin. The participants were classified as having a sedentary lifestyle if they answered "no" to all questions about engaging in any of the following activities during the past month: jogging or running, cycling, swimming, aerobic exercise, other dances, bodybuilding, garden or yard work, weightlifting, or other sports [16]. BMI is the ratio of a person's weight to their height. It was calculated by dividing weight (kg) by the square of height (sqm) [17]. The poverty-income ratio (PIR) was classified as low (< 1.3), moderate (1.3–3.5), and high (> 3.5) [18]. We also collected laboratory test data, such as triglyceride, cholesterol, HDL, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), HbA1c, and glucose levels. The NFS and FIB-4 indices for evaluating liver fibrosis were calculated as follows: NFS(19) = − 1.675 + 0.037 × age (years) + 0.094 ×BMI (kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelets(× 10^9/L) − 0.66 × albumin (g/dL), FIB-4 index [20] = [age(years) × AST (U/L)]/[platelets (×10^9/L) × ALT (U/L) ^(1/2)].

Ascertainment of mortality

We used public use-related mortality files provided by the NCHS to record cases of all-cause mortality, cardiovascular mortality, and diabetes mortality among the participants. These files use probabilistic matching algorithms to integrate data from survey participants with death certificate records from the NDI. Follow-up mortality data were updated as of December 31, 2019. All-cause mortality was the sum of deaths from all specific causes. The specific cause of death was determined according to the tenth edition of the International Classification of Diseases, with cardiovascular death codes I00-I09, I11, I13, and I20-I51 and diabetes death codes E10-E14.

Statistical analysis

Continuous variables in the table of baseline characteristics are expressed as medians (interquartile ranges), and categorical variables are expressed as frequencies and percentages. The Mann−Whitney test was used for continuous variables, and the chi-square test was used for categorical variables. Cox proportional hazard models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the NHHR and all-cause and cause-specific mortality in patients with MASLD. The choice of covariates was based on previous literature [21–25] as well as clinical experience. First, the demographic characteristics were adjusted for to establish Model 2. Adjustments for unhealthy habits and chronic diseases were then made on the basis of demographic characteristics. We used two models with progressive degrees of adjustment: Model 1 was not adjusted. Model 2 was adjusted for age, sex, race, education level, marital status, and income; Model 3 was further adjusted for smoking status, sedentary lifestyle status, diabetes status, and hypertension status on the basis of Model 2. All the candidate covariates were included in the model at the same time to avoid the randomness of the stepwise approach. To further explore the dose−response correlation between the NHHR and MASLD for all-cause mortality and cause-specific mortality, we used a restricted cubic spline (RCS) regression model. Before data analysis, we determined whether a nonlinear relationship existed by a nonlinear test, so RCS analysis was performed. According to Harrell's advice in the Regression Modelling Strategies textbook [26], 5 knots is a better choice when the sample size is greater than 100. Because the sample size in this study was more than 100, the following nodes were selected: 5%,27.5%,50%,72.5% and 95%. In addition, we examined the associations between the NHHR and all-cause and cause-specific mortality in this population according to age, sex, race, smoking status, diabetes status, hypertension status, BMI, FIB-4 index, and sedentary lifestyle subgroups. All the data in this study were analysed using R software (version 4.2.3; https://www.r-project.org/).

Results

Baseline characteristics of patients with MASLD

In the 1988–1994 NHANES database, a total of 3,155 patients were diagnosed with MASLD, of whom 1,381(43.8%) died and 1,774 (56.2%) survived. Males accounted for the majority (51.6%) of patients in the deceased group, while in the surviving group, females accounted for the majority (57.0%). In the deceased group, males (51.6%), smokers (61.3%), diabetes patients (72.8%) and hypertension patients (71.5%) accounted for the majority, while in the surviving group, females (57.0%), nonsmokers (57.6%), nondiabetes patients (55.2%) and nonhypertension patients (59.8%) accounted for the majority. Compared with the surviving group, the deceased group was older, less educated, had a lower marriage rate, was more sedentary, had a higher percentage of smokers, had larger waist circumferences, and had higher rates of diabetes and high blood pressure (All $P<0.001$). In addition, the serum triglyceride, cholesterol, ALT, ALB, and glucose indices and the NFS and FIB-4 indices were higher. Moreover, the NHHR value was higher (all $P<0.001$). In particular, non-Hispanic white patients (45.0%) accounted for the largest proportion of patients in the deceased group, while Mexican-American patients (42.5%) accounted for the largest proportion in the surviving group (all $P<0.001$). At the same time, in terms of income, the proportion of middle-income patients in both groups was the largest, at 46.30% and 44.0%, respectively (All $P<0.001$). However, there were no significant differences in BMI, HDL levels, and AST levels between the two groups (Table 1).

Table 1 Baseline characteristics of participants

Characteristics	Deceased	Alive	*P* value
	N = 1381	*N* = 1774	
Sex, n (%)			<0.001
Male	712 (51.60)	763 (43.00)	
Female	669 (48.40)	1011 (57.00)	
Age (year)	58.51 ± 12.54	39.66 ± 12.08	<0.001
Race, n (%)			<0.001
Non-Hispanic white	621 (45.00)	524 (29.50)	
Non-Hispanic black	341 (24.70)	409 (23.10)	
Mexican-American	379 (27.40)	754 (42.50)	
Other race	40 (2.90)	87 (4.90)	
Education level, n (%)			<0.001
< High school	751 (54.40)	674 (38.00)	
High school	382 (27.70)	595 (33.50)	
College	248 (18.00)	505 (28.50)	
PIR, n (%)			<0.001
Low	470 (34.00)	654 (36.90)	
Moderate	640 (46.30)	781 (44.00)	
High	271 (19.60)	339 (19.10)	
Married, n (%)	1298(70.4)	1098(79.5)	<0.001
Sedentary lifestyle, n (%)			<0.001
Negative	1022 (74.00)	1374 (77.50)	
Positive	359 (26.00)	400 (22.50)	
Smoke, n (%)			<0.001
Negative	535 (38.70)	1021 (57.60)	
Positive	846 (61.30)	753 (42.40)	
Waist circumference (cm)	104.03 ± 14.10	98.77 ± 15.35	<0.001
BMI	30.40 ± 6.38	30.05 ± 6.60	0.138
Diabetes, n (%)			<0.001
Negative	376 (27.20)	979 (55.20)	
Positive	1005 (72.80)	795 (44.80)	
Insulin (uU/mL)	18.80 ± 20.89	16.05 ± 14.64	<0.001
Hypertension, n (%)			<0.001
Negative	394 (28.50)	1060 (59.80)	
Positive	987 (71.50)	714 (40.20)	
Triglycerides (mg/dL)	194.25 ± 121.87	173.19 ± 127.40	<0.001
Cholesterol (mg/dL)	219.58 ± 44.16	204.12 ± 43.00	<0.001
HDL-C(mg/dL)	46.28 ± 15.13	45.59 ± 12.83	0.176
AST (U/L)	24.17 ± 17.48	24.20 ± 18.29	0.959
ALT (U/L)	20.35 ± 16.84	24.52 ± 23.19	<0.001
ALB (g/dL)	4.07 ± 0.36	4.14 ± 0.37	<0.001
HbA1c (%)	6.28 ± 1.69	5.56 ± 1.16	<0.001
Glucose (mg/dL)	123.58 ± 60.59	102.27 ± 39.53	<0.001
NHHR	4.14 ± 1.78	3.81 ± 1.70	<0.001
NFS	-0.66 ± 1.56	-2.11 ± 1.45	<0.001
FIB-4	0.45 ± 0.35	0.25 ± 0.14	<0.001

PIR, poverty impact ratio; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; NFS, NAFLD Fibrosis Score, FIB-4, fibrosis-4 index

Association between the NHHR and mortality risk in patients with MASLD

To explore the relationship between the NHHR and mortality risk, we used Cox proportional hazard regression models to estimate their independent associations. In unadjusted Model 1, we found that the NHHR was significantly positively associated with all-cause mortality (HR: 1.09, 95% CI: 1.06–1.12) and cardiovascular mortality (HR: 1.03, 95% CI: 0.91–1.17) in patients with MASLD. After adjusting for age, sex, race, education level, marital status and income in Model 2, the NHHR was only related to cardiovascular mortality (HR: 1.07, 95% CI: 1.01–1.13). The NHHR was not significantly associated with all-cause mortality, cardiovascular mortality or diabetes mortality in Model 3. Therefore, the NHHR had no significant relationship with all-cause mortality ($P=0.593$), cardiovascular mortality ($P=0.085$), or diabetes mortality ($P=0.200$) (Table 2).

Nonlinear trends in NHHR and mortality among patients with MASLD

We further investigated the relationship between the NHHR and mortality in patients with MASLD using RCS-fitted Cox proportional risk models. In Model 1, Model 2 and Model 3, we found a significant nonlinear association between the NHHR and all-cause mortality (nonlinear $P=0.002$, 0.018, 0.004, respectively)

Table 2 HRs of NHHR indices for all-cause and cause-specific mortality with MASLD

Outcome	Model 1		Model 2		Model 3	
	HR (95%CI)	*P*	HR (95%CI)	*P*	HR (95%CI)	*P*
All-cause	1.09 (1.06 ~ 1.12)	< 0.001	1.02 (0.99 ~ 1.06)	0.168	1.01 (0.98 ~ 1.04)	0.593
Cardiovascular	1.03 (0.91 ~ 1.17)	< 0.001	1.07 (1.01 ~ 1.13)	0.017	1.06 (0.99 ~ 1.13)	0.085
Diabetes	0.95 (0.85 ~ 1.08)	1.000	0.95 (0.85 ~ 1.08)	0.450	0.92 (0.80 ~ 1.05)	0.200

Model 1: Non-adjusted

Model 2: Adjusted for age, gender, race, education, marital status, income

Model 3: Adjusted for smoking, sedentary lifestyle, diabetes, and hypertension on the basis of model2. HR: Hazard ratio; CI: Confidence interval

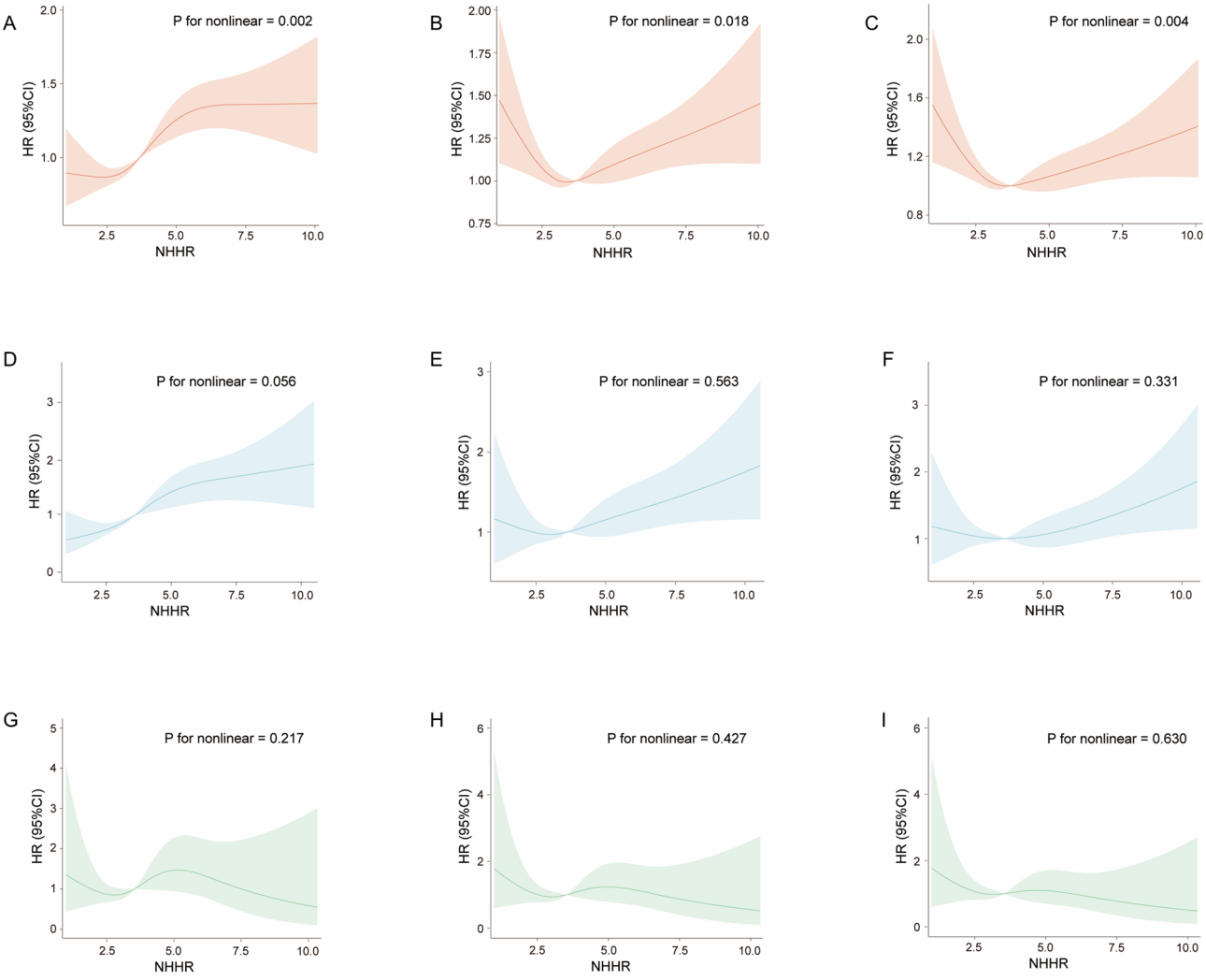

Fig. 1 Nonlinear relationships between the NHHR and mortality according to the three models. The solid lines in the figure represent the HRs, and the shaded regions represent the 95% CIs. Model 1 was not adjusted. Model 2 was adjusted for age, sex, race, education level, marital status, and income; Model 3 was further adjusted for smoking status, sedentary lifestyle status, diabetes status, and hypertension status on the basis of Model 2. (**A**) Model 1 and all-cause mortality (**B**) Model 2 and all-cause mortality (**C**) Model 3 and all-cause mortality (**D**) Model 1 and cardiovascular mortality (**E**) Model 2 and cardiovascular mortality (**F**) Model 3 and cardiovascular mortality (**G**) Model 1 and diabetes mortality (**H**) Model 2 and diabetes mortality (**I**) Model 3 and diabetes mortality

(Fig. 1A-C). However, although the *p* value of Model 1 and cardiovascular mortality was close to 0.05, it was not statistically significant (Fig. 1D). Moreover, the analysis showed that there was no significant association between the NHHR and cardiovascular mortality in Models 2 or 3 (nonlinear $P = 0.563$, 0.331, respectively) (Fig. 1E-F). In addition, it was ascertained that there was no pronounced nonlinear correlation between diabetes mortality and the NHHR in Model 1, Model 2, or Model 3 (nonlinear $P = 0.217$, 0.427, 0.630, respectively) (Fig. 1G-I).

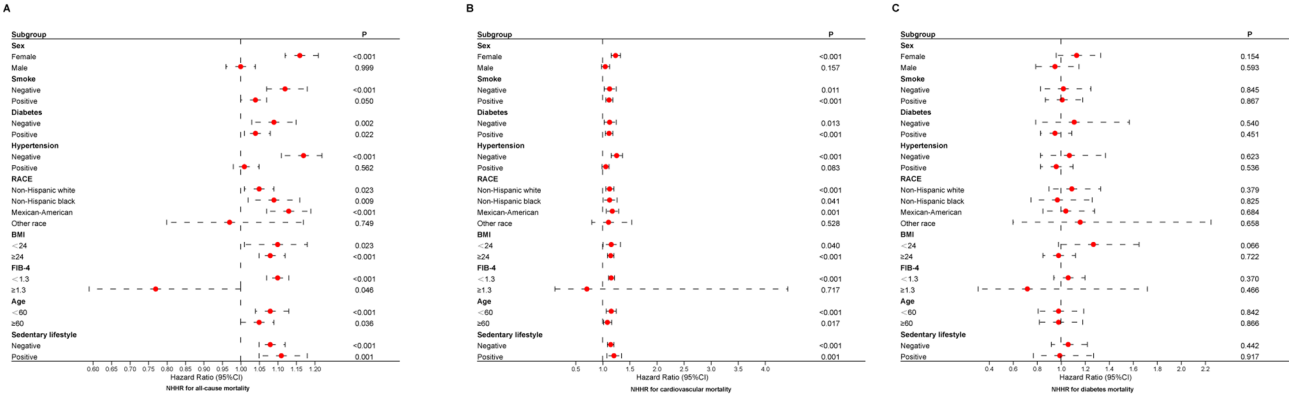

Fig. 2 Subgroup analysis of the association between the NHHR and MASLD mortality. Red dots indicate the HR, and the bars on both sides of the dots indicate the 95% CI of the HR. (**A**) NHHR and all-cause mortality. (**B**) NHHR and cardiovascular mortality. (**C**) NHHR and diabetes mortality

Subgroup analysis of the NHHR and mortality in patients with MASLD

To further study the association between the NHHR and mortality in different populations, we stratified the MASLD population according to age, sex, race, smoking status, hypertension status, diabetes status, sedentary lifestyle status, BMI and the FIB-4 index. Stratified analyses showed that the NHHR was associated with an increase in all-cause mortality and cardiovascular mortality among female, non-hypertension patients, Non-Hispanic white patients, Non-Hispanic black patients, Mexican-American patients. Alternatively, the association of NHHR with cardiovascular mortality was more likely to occur in the population without advanced fibrosis. (Fig. 2A-B). There was no significant association of the NHHR with diabetes mortality (Fig. 2C). These results suggest that NHHR better predicted both all-cause and cardiovascular mortality in female, non-hypertensive patients, and specific racial/ethnic groups, but its predictive value for mortality in non-advanced liver fibrosis was confined to cardiovascular mortality.

Discussion

Based on a large-scale prospective cohort study, we preliminarily observed the association between the NHHR and all-cause mortality, cardiovascular mortality, and diabetes mortality in patients with MASLD. Our study showed that NHHR showed a significant nonlinear association with all-cause mortality. In patients without advanced liver fibrosis, the association between the NHHR and cardiovascular mortality was more pronounced. This study elucidates the correlation between the NHHR and all-cause and cardiovascular mortality, which can provide a reference for clinical patient prevention and follow-up and a theoretical basis for formulating intervention strategies to reduce MASLD-related deaths.

Patients with MASLD often present with atherosclerotic dyslipidaemia, which is characterized by hypertriglyceridaemia, low levels of HDL-C and low-density lipoprotein cholesterol (LDL-C) particles that are smaller and denser than normal [27]. MASLD is often accompanied by insulin resistance and lipid metabolism disorders [28]. In recent years, many studies have revealed that the NHHR is closely related to diseases that are related to dyslipidaemia. A study showed a substantial correlation between elevated NHHR values in adults and a higher likelihood of kidney stone occurrence and recurrence [13]. Among adults with diabetes or prediabetes in the United States, the NHHR had a U-shaped association with all-cause mortality and an L-shaped association with cardiovascular mortality [29]. Cheng et al. found that increased NHHR values were strongly associated with increased gallstone incidence in people aged ≤ 50 years [30]. In addition, some studies have suggested that the non-HDL-c/HDL-c ratio might be a feasible predictor for NASH [14]. In addition, the study of Yang et al. showed that the NHDL-C/HDL-C ratio was positively correlated with NAFLD in Chinese children and adolescents [31]. However, since NAFLD was renamed MASLD, no studies have been conducted to explore the relationship between the NHHR and MASLD. Our study found NHHR was found to be significantly associated with MASLD mortality, which is basically consistent with the conclusions of previous studies on NAFLD. Nevertheless, the pathogenesis of MASLD is complex, and lipotoxicity and insulin resistance are not the only conditions that can lead to the development of MASLD. According to previous studies, gut microbiota imbalance and the consumption of a pro-inflammatory diet can also drive the chronic inflammation caused by MASLD. MASLD is usually accompanied by severe gut microbiota imbalance and the evolution of some pathogenic factors, especially in the late stage of the disease process [32]. Patients with advanced liver fibrosis and MASLD have serious intestinal flora imbalance, which is accompanied by the emergence of pathogenic phyla such as Proteobacteria and *Escherichia coli* [32].

In addition, specific dietary components may induce and promote low-grade inflammation, including in the liver, and may promote a proinflammatory state when an anti-inflammatory diet is deficient or underconsumed [33]. Witkowski reported that consumption of dietary components such as palmitic acid or trans-fatty acids may lead to elevated circulating levels of trimethylamine N-oxide, which is associated with atherosclerosis and low-grade systemic inflammation [34]. Moreover, MASLD is a multifactorial disease, and up to 50% of its relative risk is attributed to genetic susceptibility [35, 36]. Genetic dyslipidaemia is also an important factor that cannot be ignored. Previous studies have shown that variants in the PNPLA3, TM6SF2, LYPLAL1, and GCKR genes are associated with elevated liver fat levels [37]. The NHHR in this study was based on blood lipid levels, which may ignore other MASLD factors, such as dysbiosis and diet, and may underestimate MASLD mortality. In the future, we will develop other more comprehensive indicators to assess MASLD mortality and provide more accurate estimations.

Non-high-density lipoprotein cholesterol (non-HDL-C) refers to the sum of cholesterol contained in lipoproteins other than high-density lipoprotein, mainly including LDL-C and very-low-density lipoprotein cholesterol [38]. The present study revealed the importance of non-HDL-C over LDL-C in predicting the risk of atherosclerotic cardiovascular disease. Moreover, non-HDL-C was a better risk predictor in patients with atherosclerotic dyslipidaemia with metabolic disorders, type 2 diabetes, and obesity [39]. A prospective cohort study of 213 subjects from the general population without liver disease reviled that the non-HDL-C level was a better predictor of NAFLD than total cholesterol, LDL cholesterol, triglyceride, and HDL levels. Patients with non-HDL-C levels < 130 mg/dl did not develop NAFLD, while 20.8% of patients with levels of 130–160 mg/dl developed NAFLD and 24.6% of patients with levels > 160 mg/dl developed NAFLD [40]. Another study found that the lowest levels of non-HDL-C at 2 months and 1 year in patients with myocardial infarction were associated with better outcomes [41]. Therefore, determine the NHHR value can help to predict the occurrence of adverse events in MASLD at an early stage, provide more ideas for clinicians to make decisions, and reduce the rate of MASLD mortality, thereby reducing the global economic burden.

The NHHR is the ratio of non-HDL cholesterol to HDL cholesterol. The NHHR was shown to be associated with mortality in patients with MASLD in this study, and the specific mechanism may be related to the following factors. First, the disruption of free cholesterol accumulation and homeostasis in the liver is closely related to the pathogenesis of MASLD. Dysregulation of cholesterol homeostasis leads to the accumulation of free cholesterol in the liver, which contributes to the development of MASLD and atherosclerosis [42]. In addition, free cholesterol can activate and accumulate in Kupffer cells, leading to immune activation, oxidative stress, inflammation, and cell death [43]. Moreover, free cholesterol and its derivatives trigger various intracellular reactions that lead to lipotoxic stress in the mitochondria and endoplasmic reticulum, leading to hepatic steatosis and promoting ongoing hepatocyte death and liver injury [44]. This disorder of lipid metabolism may exacerbate liver fat accumulation and inflammation and promote the progression of MASLD, thereby increasing the risk of cardiovascular disease and liver-related complications. The disorder of liver cholesterol homeostasis and accumulation of free cholesterol can promote the formation of atherosclerotic plaques and increase the incidence of cardiovascular events such as myocardial infarction and stroke, leading to increased mortality. Second, increased cholesterol synthesis and decreased absorption are associated with insulin resistance [45], which is one of the core pathophysiological mechanisms of MASLD. Insulin resistance may further aggravate the manifestations of metabolic syndrome (obesity, hypertension, hyperglycaemia, etc.), thereby increasing all-cause mortality, cardiovascular mortality, and diabetes mortality [46]. In addition, HDL may accelerate liver fibrosis and extrahepatic organ damage by promoting systemic inflammation and oxidative stress [46]. Chronic inflammation and oxidative stress are important driving factors for the progression of MASLD to liver cirrhosis and liver cancer and are also closely related to the occurrence and development of cardiovascular diseases. HDL plays a very important role in the organism. It not only acts as a barrier to resist chemical and biological invasion but can also repair tissue damage by regulating inflammation, promoting signal transmission and metabolic remodelling to ensure the normal operation of the body. Similar to reversible cholesterol transport, after cholesterol accumulation, macrophages exert multiple proinflammatory activities that are inhibited by HDL-mediated cholesterol efflux [47]. It also regulates the inflammatory effects of innate and adaptive immune cells, supports the integrity and function of the endothelial barrier, and stimulates angiogenesis [47]. Furthermore, the proteome of HDL is rich in proteases and protease inhibitors, which can regulate platelet aggregation, coagulation, fibrinolysis, complement activation and tissue degradation. It helps to heal wounds and maintain body health during injury, infection or inflammation [48]. Therefore, when HDL metabolism is disordered, it may lead to the release of inflammatory factors, cause cell damage and apoptosis, promote systemic inflammatory response, and lead to the occurrence or progression of autoimmune diseases, cardiovascular diseases, diabetes

and infectious diseases, thereby increasing the risk of mortality in patients.

In summary, we conducted this prospective large-scale cohort study to investigate the association of the NHHR with all-cause and cause-specific mortality in patients with MASLD. Our study showed a nonlinear correlation between the NHHR and all-cause mortality in patients with MASLD. In addition, NHHR appears to have greater predictive power for cardiovascular mortality in MASLD without progressive liver fibrosis. We believe that this finding may be related to the reduced protein and cholesterol synthesis capacity in patients with decompensated cirrhosis, as indicated by significantly lower low-density lipoprotein (LDL), high-density lipoprotein (HDL), and cholesterol content, as compared with liver-healthy controls [49, 50]. At this time, NHHR may not reflect traditional cardiovascular risk. In addition, patients with advanced liver disease were more likely to die of liver failure or infection than cardiovascular events, leading to diminished predictive value of NHHR. Moreover, the systemic inflammation that accompanies advanced fibrosis may alter lipoprotein function and bias the association of NHHR with mortality. At the same time, this study could not dynamically monitor changes in NHHR or subclassify causes of death. Therefore, further validation of the clinical significance of NHHR in advanced liver disease is needed in the future. A 2018 study explored the association between the NHHR and the risk of developing NASH/NAFLD and the prevalence of NASH [14]. In contrast, our study, which focused on the relationship between the NHHR and all-cause and cause-specific mortality, was based on an analysis of data from the NHANES III (1988–1994), in which participants were followed for up to 6 years, and the follow-up data cover demographic, epidemiological and other information. In addition, our research object was MASLD after the diagnostic criteria were changed. Our study provides more information on the relationship between the NHHR and MASLD mortality. Although recent studies have found that the NHHR plays a predictive role in the occurrence and development of a variety of diseases, its specific mechanism of action is not yet clear. It is necessary to further explore the specific mechanism of the NHHR in the future.

However, there are some limitations to our study. First, the diagnosis of SLD in this study was determined based on ultrasound findings because there was no liver pathology data in the database; therefore, it SLD was not determined based on liver pathology, which may lead to inaccurate diagnosis due to the subjectivity of ultrasound. Moreover, due to the lack of imaging data such as FibroScan data in the NHANES III database, this study used the FIB-4 and NFS indices to noninvasively evaluate advanced liver fibrosis in patients with MASLD according to the AASLD [51] guidelines. Despite the advantages of noninvasive methods in terms of convenience and cost-effectiveness, the choice of diagnostic threshold and influence of factors such as age and platelet count can lead to possible diagnostic bias compared with imaging methods. In future studies, we will integrate imaging data to increase the accuracy of liver fibrosis assessment and thus enhance the reliability of our findings. Second, this study included only data from the US, which will need to be verified in a larger population. Third, in this study, we focused on the association of the NHHR with all-cause mortality, cardiovascular disease, and diabetes-related mortality, as these are the most common complications and causes of death in patients with MASLD. However, due to the limited data in this study, the relationship between the NHHR and mortality of other disease-specific factors in patients with MASLD was not further explored. We plan to expand the scope of data collection in future studies to include detailed information on liver disease, cancer, and other metabolism-related diseases to more comprehensively evaluate the outcomes of patients with MASLD. Fourth, in this study, because of limitations in data sources, we did not obtain detailed information on the use of lipid-lowering medications, such as statins, fibrates, and niacin. This may have led us to fail to fully account for the potential effects of these drugs on the results of our analyses. Data were also not systematically collected at each visit, which makes it difficult to directly assess the effect of changes in mortality and associations over time. In future studies, we plan to expand our data collection by establishing our own database to include detailed information on the use of lipid-lowering drugs, including the type, dose, and duration of use, to more accurately assess the effect of drugs on cardiovascular outcomes. At the same time, a long-term follow-up mechanism was established to collect relevant data at each visit, and the effects of time-varying factors on mortality and correlation were analysed in depth. Finally, as this was an observational study, we were unable to elucidate a causal association between the NHHR and mortality in patients with MASLD.

Conclusions

The NHHR was significantly associated with all-cause mortality in U.S. adults with MASLD. In addition, the association between NHHR and cardiovascular mortality was more pronounced among participants without advanced fibrosis. Our findings suggest that the NHHR is useful in assessing the risk of death and prognosis of MASLD in adults, helping clinicians to provide more individualized care.

Abbreviations

ALB	Albumin
ALT	Alanine aminotransferase

AST	Aspartate aminotransferase
BMI	Body mass index
CI	Confidence intervals
CKD	Chronic kidney disease
CVD	Cardiovascular disease
FBG	Fasting Blood Glucose
HCC	Hepatocellular carcinoma
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratios
LDL-C	Low-density lipoprotein cholesterol
MASLD	Metabolic dysfunction-associated steatotic liver disease
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NDI	National death index
NHANES	III: National health and nutrition examination survey
NHHR	Non-high-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio
Non-HDL-C	Non-high-density lipoprotein cholesterol
OSA	Obstructive sleep apnea
PIR	Poverty-income ratio
RCS	Restricted cubic spline
SLD	Steatotic liver disease
T2DM	Type 2 diabetes mellitus
UNOS	United network for organ sharing
WC	Waist circumferencecm

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Author contributions
YZ designed and drafted the manuscript and analyzed the data. PL, YT and JW collected and analyzed the data. SG and YF have revised manuscript. KW has contributed to the interpretation of data and the critical revision of articles on important intellectual content.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All survey protocols were approved by the National Center for Health Statistics Ethics Review Board in USA. All participants provided written informed consent before participation.

Consent for publication
All authors have reviewed and approved the final version of the manuscript.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Hepatology, Qilu Hospital of Shandong University, Wenhuaxi Road 107#, Jinan, Shandong 250012, China
[2]Hepatology Institute of Shandong University, Jinan 250012, China

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