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ORIGINAL ARTICLE

AST to ALT Ratio as a Potential Biomarker for Type 2 Diabetes: Findings from the NHANES Database

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ABSTRACT

Background: **The aspartate transaminase to alanine aminotransferase ratio (AST/ALT) has been proposed as a potential biomarker for metabolic dysfunction, but its association with type 2 diabetes mellitus (T2DM) remains controversial. This study aimed to investigate the relationship between AST/ALT ratio and risk of T2DM using a nationally representative sample.**
Methods: **We analyzed data from the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2004, collecting details on their T2DM status, AST/ALT ratio, and several other essential variables. There were 12,527 participants, with 13.9% (1,746/12,527) who experienced T2DM. Logistic regression, curve fitting, interaction effects were utilized to investigate the association between AST/ALT ratio and T2DM.**
Results: **There was a substantial difference in AST/ALT ratios between the groups, with T2DM patients exhibiting significantly lower values ($p < 0.001$). Logistic regression analysis revealed a negative association between AST/ALT ratio and T2DM, even after adjusting for the confounding variables (odds ratio: 0.28, 95% confidence interval: 0.25 - 0.32, $p < 0.001$). In addition, curve fitting after adjusting for all the confounding variables showed that there was a linear relationship between AST/ALT ratio and T2DM (p for non-linearity = 0.107). AST/ALT ratio was negatively correlated with T2DM.**
Conclusions: **The AST/ALT ratio was associated with a lower risk of T2DM in a linear pattern, suggesting its potential role as a clinical indicator of metabolic risk. Further prospective studies are needed to clarify causality.**
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KEYWORDS

AST, ALT, type 2 diabetes mellitus, linear association

INTRODUCTION

Originally utilized as a clinical measure of liver function, the AST/ALT ratio has emerged as a potential biomarker in metabolic dysregulation, particularly in relation to type 2 diabetes mellitus (T2DM) [1,2]. Evidence is accumulating that dysregulated liver enzymes, especially ALT and AST, may serve as indicators of metabolic dysfunction and fatty liver, both of which are pivotal in T2D pathogenesis [3,4]. While ALT is primarily associated with liver fat accumulation and insulin resistance, AST has broader tissue distribution, including cardiac and skeletal muscle. Therefore, this ra-

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tio of AST/ALT could act as a combined marker reflecting liver and systemic metabolic disturbances that contribute to diabetes pathogenesis [5,6]. The nationally representative NHANES dataset, containing standardized biochemical and clinical assessments, enables rigorous examination of this potential biomarker relationship [7,8]. Previous studies have examined individual liver enzymes in relation to T2DM, but the AST/ALT ratio's predictive utility remains less understood. Investigating this ratio could enhance early diabetes risk stratification, particularly in individuals with underlying metabolic dysregulation.

This study aims to evaluate the association between the AST/ALT ratio and T2DM risk using NHANES data. By examining this comprehensive national dataset, our study addresses the translational potential of this biomarker for clinical diabetes risk assessment.

MATERIALS AND METHODS

Participant Recruitment

The study was based on cross-sectional data from NHANES (1999 - 2004), a nationally representative survey administered by the CDC [9,10]. The National Health and Nutrition Examination Survey (NHANES) employs a complex sampling design involving multiple stages and stratified probability sampling to assess the health and nutritional characteristics of the non-institutionalized US population. This nationwide survey collects extensive demographic and health information through household interviews, clinical examinations, and laboratory analyses conducted via mobile assessment centers. Since the current research utilized previously collected NHANES datasets, institutional review board (IRB) approval was not required for this secondary analysis [11]. The publicly available dataset can be accessed through the official NHANES portal (http://www.cdc.gov/nchs/nhanes.htm). Our analysis included adult participants aged more than 20 years who completed the survey interview. We excluded pregnant individuals and those with missing diabetes mellitus (DM) status or liver enzyme (AST/ALT) measurements. Finally, a total of 12,527 participants were included in the final analysis (Figure 1), 1,746 with DM, 10,781 without DM.

The study utilized data from the NHANES program, following established protocols and technical guidelines. Demographic characteristics (including gender, age, and race), clinical examinations (such as blood pressure, BMI), lifestyle factors (like smoking, alcohol drinking status, and comprehensive laboratory tests (covering hematological parameters, metabolic markers, liver enzymes, and lipid profiles) were analyzed. All laboratory analyses were conducted at a certified facility in Minnesota, with measurements standardized according to international units. The complete dataset and methodology are publicly accessible through the NHANES online repository.

Evaluation Criteria

Definition of diabetes mellitus (DM): Diabetes mellitus was defined according to internationally recognized diagnostic criteria, consistent with established guidelines and prior literature [12]. Diabetes was defined by meeting any of the following criteria: physician-diagnosed diabetes; 2-hour plasma glucose ≥ 11.1 mmol/L during an oral glucose tolerance test (OGTT); random plasma glucose ≥ 11.1 mmol/L; fasting plasma glucose ≥ 7.0 mmol/L; glycated hemoglobin (HbA1c) > 6.5%; use of diabetes medications or insulin. Based on established criteria from prior studies, smoking status was classified into three groups: Never smokers (individuals who smoked fewer than 100 cigarettes in their lifetime), current smokers (those actively smoking at the time of assessment), former smokers (individuals who had smoked more than 100 cigarettes but subsequently quit). Alcohol consumption status was classified as follows: Never drinkers: individuals who consumed fewer than 12 alcoholic drinks in their lifetime. Former drinkers: Those who either drank ≥ 12 drinks in at least one year but abstained in the past year, or had a lifetime intake of ≥ 12 drinks despite not drinking in the previous year. Current drinkers: Participants who consumed ≥ 12 drinks in any single year and reported alcohol use within the past year [13].

Evaluation of Body Mass Index (BMI): Body mass index (BMI) was derived by dividing an individual's weight (measured in kilograms) by their height (in meters) squared. Based on World Health Organization (WHO) standards, participants were categorized as follows: normal weight (BMI 18.5 - 24.9 kg/m²), overweight (BMI 25.0 - 29.9 kg/m²), or obese (BMI ≥ 30.0 kg/m²) [14].

The identification of pre-existing conditions (hypertension and coronary heart disease) relied on self-reported questionnaire responses regarding physician-diagnosed medical history [15].

Covariate selection criteria

The assessed covariates encompassed: Demographic information, laboratory test results, clinical examination data, health behavior indicators. The selection of covariates was guided by: Basic demographic characteristics; clinically relevant factors based on medical expertise; established risk factors for DM identified in prior studies; variables whose inclusion altered the base regression model coefficients by > 10%, indicating meaningful confounding effects. The analyses were conducted in R software (version 4.2.3), utilizing multiple packages including gtsummary, survey, rms, ggplot2, and forestplot. All participant data underwent descriptive analysis, with statistical significance defined as $p < 0.05$ (two-tailed tests).

Table1. Comparison of baseline metrics between participants grouped according to DM.

Variables	Total	No	Yes	p
	(n = 12,527)	(n = 10,781)	(n = 1,746)	
Gender, n (%)				
Male	6,312 (50.4)	5,396 (50.1)	916 (52.5)	0.062
Female	6,215 (49.6)	5,385 (49.9)	830 (47.5)	
Age (years)	51.0 ± 18.8	49.1 ± 18.8	62.8 ± 13.8	< 0.001
BMI (kg/m^2)	28.3 ± 6.2	27.8 ± 5.9	31.3 ± 6.8	< 0.001
Race, n (%)				
Non-Hispanic white	6,357 (50.7)	5,657 (52.5)	700 (40.1)	< 0.001
Non-Hispanic black	2,364 (18.9)	1,962 (18.2)	402 (23)	
Mexican American	2,819 (22.5)	2,328 (21.6)	491 (28.1)	
Other	987 (7.9)	834 (7.7)	153 (8.8)	
Hypertension, n (%)	3,371 (27.2)	2,429 (22.8)	942 (54.5)	< 0.001
CHD, n (%)	584 (4.7)	391 (3.6)	193 (11.2)	< 0.001
Smoking, n (%)				
Never	6,305 (50.4)	5,500 (51.1)	805 (46.2)	< 0.001
Former	3,386 (27.1)	2,746 (25.5)	640 (36.7)	
Current	2,820 (22.5)	2,523 (23.4)	297 (17)	
ALB, (g/L)	43.0 ± 3.4	43.2 ± 3.3	41.7 ± 3.5	< 0.001
BUN, (mmol/L)	5.1 ± 2.2	4.9 ± 1.9	6.1 ± 3.3	< 0.001
Cholesterol, (mmol/L)	5.2 ± 1.1	5.2 ± 1.1	5.2 ± 1.2	0.098
Glucose, (mmol/L)	5.5 ± 2.0	5.0 ± 0.6	8.4 ± 4.1	< 0.001
TP (g/L)	73.9 ± 4.9	73.9 ± 4.8	73.9 ± 5.3	0.758
UA, (μmol/L)	324.3 ± 87.1	322.4 ± 85.8	336.2 ± 94.2	< 0.001
Cr, (μmol/L)	78.1 ± 44.8	76.4 ± 39.0	88.3 ± 69.9	< 0.001
Alcohol drinking status (%)				
Never	1,712 (14.6)	1,395 (13.9)	317 (19.3)	< 0.001
Former	2,445 (20.9)	1,859 (18.5)	586 (35.6)	
Current	7,556 (64.5)	6,814 (67.7)	742 (45.1)	
AST/ALT	1.1 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	< 0.001
ALP, (U/L))	71.0 (58.0, 87.0)	70.0 (57.0, 86.0)	77.0 (62.0, 95.0)	< 0.001
GGT, (U/L)	21.0 (15.0, 33.0)	21.0 (15.0, 32.0)	26.0 (19.0, 41.0)	< 0.001
TG, (mmol/L))	1.3 (0.9, 1.9)	1.2 (0.8, 1.8)	1.8 (1.2, 2.7)	< 0.001

Data presented are mean ± SD, median (IQR), or N (%).
BMI: Body Mass Index, ALB: albumin, TP: total protein, Hypertension: high blood pressure, CHD: coronary heart disease, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, TG: triglyceride, Cr: creatinine, UA: uric acid.

RESULTS

Baseline Demographic Features

This research included 31,126 participants from the NHANES dataset and took place over six years across three cycles. After applying the stringent screening criteria mentioned earlier, the final analysis comprised 12,527 participants, with a mean age of 51.0 ± 18.8 years. Among them, 1,746 had DM and 10,781 had no DM. The relevant clinical and laboratory data for the participants are presented in Table 1. There is a significant difference in age, BMI, race, hypertension, coronary heart disease (CHD), smoking, alcohol drinking status, ALP, ALB, BUN, Cr, glucose, UA, GGT, TG, and AST/ALT between the DM group and the non-DM group. However, no significant differences were observed between the two groups regarding gender, cholesterol, or total protein (TP).

Table 2. Single-variable analysis examining the relationship between interrelated variables and DM.

Variable	DM	
	OR (95% CI)	p-value
Male	1	
Female	0.91 (0.82 - 1)	0.062
Age (years)	1.04 (1.04 - 1.05)	< 0.001
BMI (kg/m^2)	1.08 (1.07 - 1.09)	< 0.001
Non-Hispanic white	1	
Non-Hispanic black	1.66 (1.45 - 1.89)	< 0.001
Mexican American	1.70 (1.5 - 1.93)	< 0.001
Other	1.48 (1.23 - 1.79)	< 0.001
Hypertension, n (%)	4.05 (3.64 - 4.49)	< 0.001
CHD, n (%)	3.34 (2.79 - 4.01)	< 0.001
Smoking, n (%) never	1	
Former	1.59 (1.42 - 1.78)	< 0.001
Current	0.80 (0.7 - 0.93)	0.002
ALB, (g/L)	0.88 (0.86 - 0.89)	< 0.001
ALP, (U/L)	1.01 (1.01 - 1.01)	< 0.001
BUN, (mmol/L)	1.22 (1.2 - 1.25)	< 0.001
Cholesterol, (mmol/L)	1.04 (0.99 - 1.09)	0.098
GGT, (U/L)	1.00 (1 - 1)	< 0.001
Glucose, (mmol/L)	5.01 (4.63 - 5.42)	< 0.001
TP (g/L)	1.00 (0.99 - 1.01)	0.758
TG, (mmol/L))	1.41 (1.36 - 1.46)	< 0.001
UA, (μmol/L)	1.00 (1 - 1)	< 0.001
Cr, (μmol/L)	1.00 (1 - 1.01)	< 0.001
Alcohol drinking status (%)		
Never	1	
Former	1.39 (1.19 - 1.62)	< 0.001
Current	0.48 (0.42 - 0.55)	< 0.001
AST/ALT	0.56 (0.48 - 0.66)	< 0.001

Data presented are mean ± SD, median (IQR), or N (%).
BMI: Body Mass Index, ALB: albumin, TP: total protein, Hypertension: high blood pressure, CHD: coronary heart disease, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, ALP: alkaline phosphatase, BUN: blood urea nitrogen, TG: triglyceride, Cr: creatinine, UA: uric acid.

Univariate Analysis of Factors associated with DM

Univariate logistic regression analysis revealed that age, BMI, race, hypertension, CHD, smoking, alcohol drinking status, ALB, ALP, GGT, BUN, glucose, TG, UA, Cr, and AST/ALT were significantly associated with diabetes mellitus (DM). Among these, age, BMI, hypertension, CHD, ALP, BUN, glucose, TG, UA, and Cr exhibited a positive association with DM risk, whereas ALB, and AST/ALT showed an inverse relationship. Notably, non-Hispanic white had a lower likelihood of DM compared to other racial groups. Relative to never-smokers, former smoking was positively correlated with DM, whereas current smoking showed an inverse relationship. Compared to never drinkers, former drinkers are positively associated with DM, while current drinkers are negatively associated with DM (Table 2).

Multivariate analysis of factors associated with AST/ALT and DM

After performing multiple imputation for missing covariates, we conducted logistic regression analyses to examine the association between AST/ALT ratio (divided into tertiles) and diabetes mellitus (DM). Four progressively adjusted models were developed, all demonstrat-

Table 3. Multivariate analysis of association between AST/ALT and DM in individuals.

Variable	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
AST/ALT	0.56 (0.51 - 0.62)	< 0.001	0.20 (0.17 - 0.22)	< 0.001	0.27 (0.24 - 0.30)	< 0.001	0.28 (0.25 - 0.32)	< 0.001
AST/ALT group								
T1 (0.06 - 0.93)	1 (Ref)		1 (Ref)		1 (Ref)		1 (Ref)	
T2 (0.93 - 1.19)	0.87 (0.82 - 0.93)	< 0.001	0.56 (0.52 - 0.61)	< 0.001	0.60 (0.56 - 0.65)	< 0.001	0.64 (0.59 - 0.70)	< 0.001
T3 (1.19 - 6.00)	0.59 (0.55 - 0.64)	< 0.001	0.28 (0.26 - 0.310)	< 0.001	0.35 (0.32 - 0.38)	< 0.001	0.37 (0.33 - 0.40)	< 0.001
p for trend		< 0.001		< 0.001		< 0.001		< 0.001

Model 1: Non-adjusted.
Model 2: Age, gender, race
Model 3: Model 2 + BMI, hypertension, CHD, smoking, alcohol using.
Model 4: Model 3 + ALB, TP, GGT, ALP, BUN, TG, Cr, UA, cholesterol.

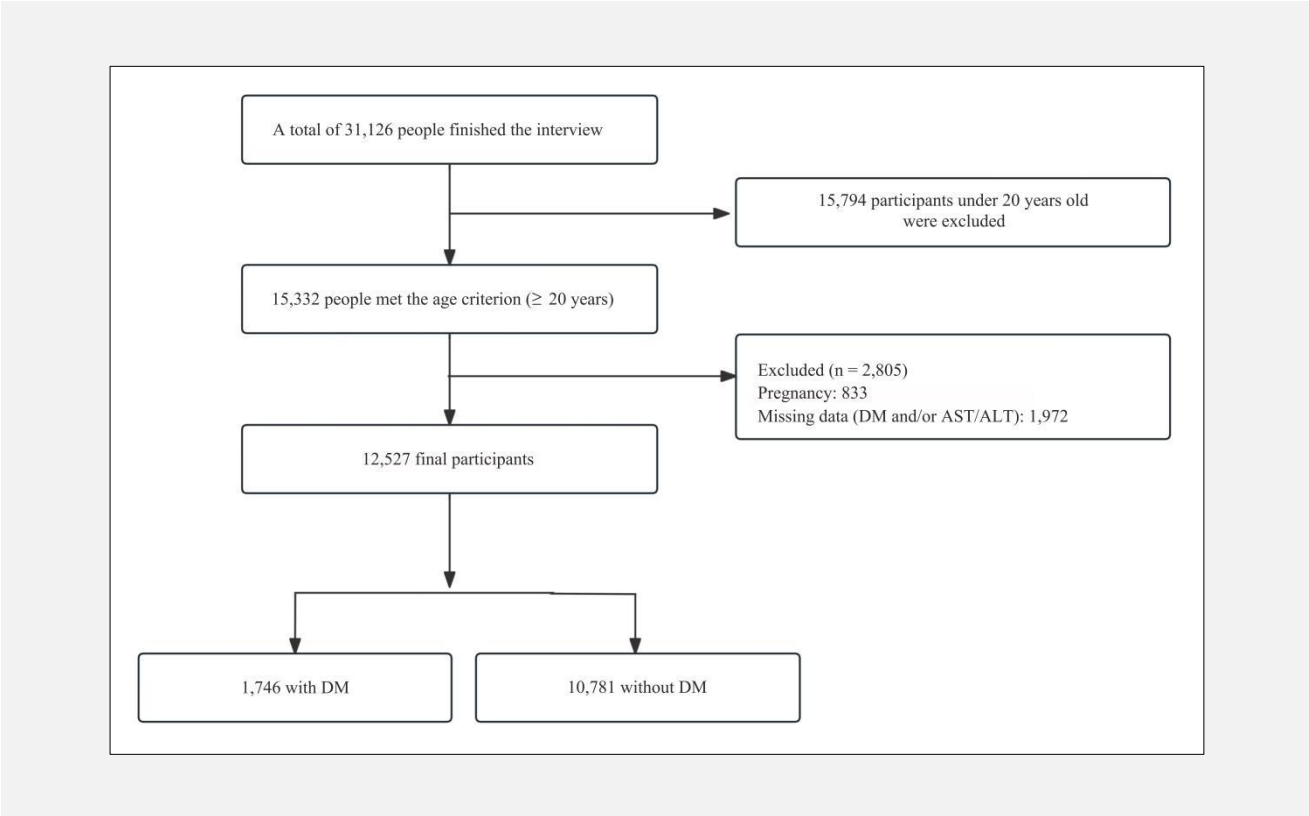

Figure 1. Participants' screening and selection flowchart.

ing a statistically significant inverse relationship ($p < 0.001$).

The odds ratios (OR) with 95% confidence intervals consistently showed decreasing DM risk with higher AST/ALT ratios across all models: Unadjusted model: OR 0.56 (0.51 - 0.62), indicating 44% lower DM risk per unit increase; partially adjusted model: OR 0.20 (0.17 - 0.22), showing 80 % risk reduction; further ad-

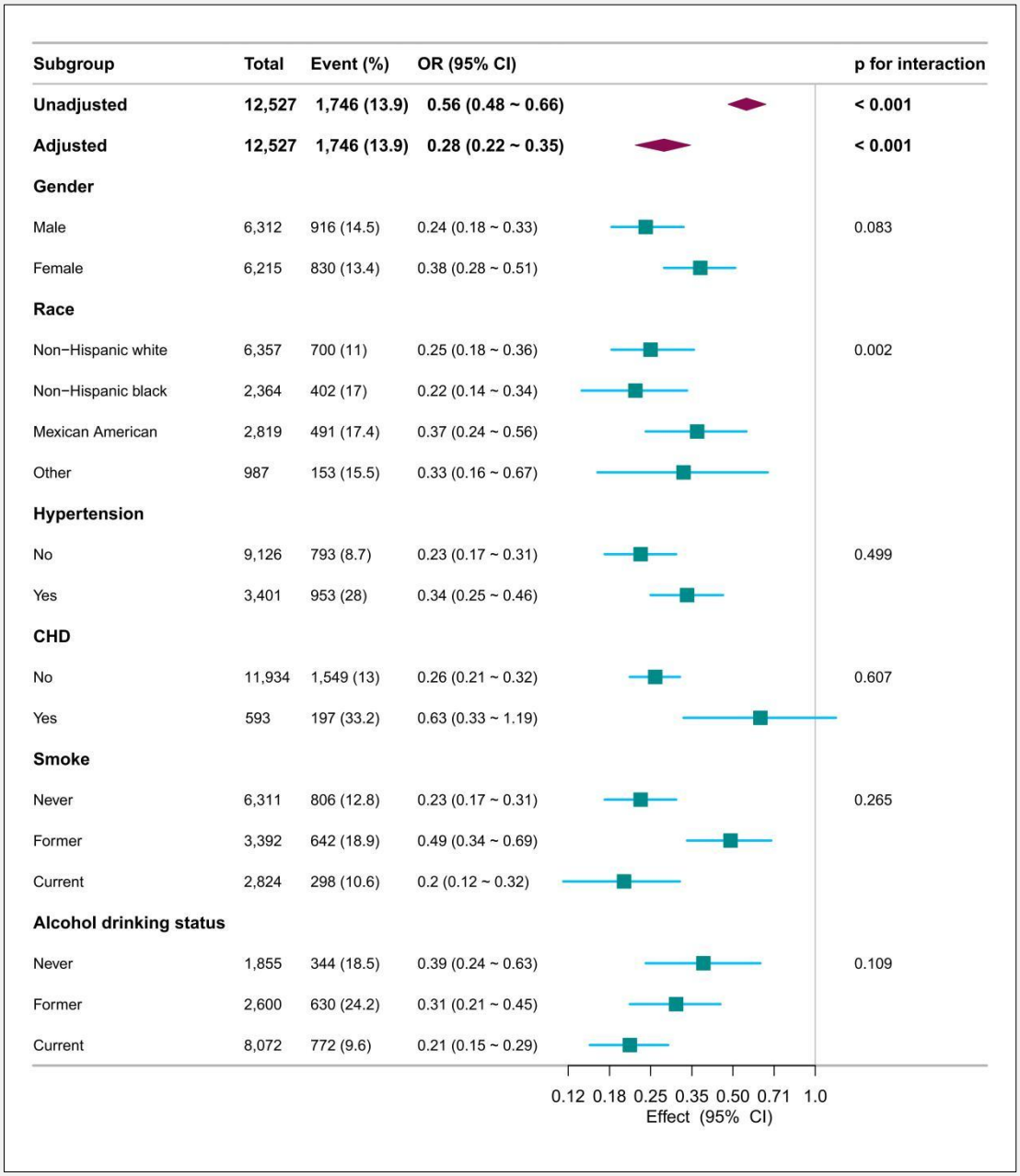

Figure 2. Forest plot analysis of liver enzyme ratio (AST/ALT) and DM.

justed model: OR 0.27 (0.24 - 0.30), representing 73% lower risk; fully adjusted model: OR 0.28 (0.25 - 0.32), maintaining 72% risk reduction. Statistical analysis revealed a highly significant association ($p < 0.001$). A consistent inverse relationship was observed between AST/ALT and DM risk in every model, with highly significant p-values (< 0.001). The consistent dose-response pattern across all models suggests AST/ALT ratio may serve as a protective factor against DM development (Table 3).

Subgroup Analysis and Curve Fitting

We explored whether there were differences in gender, race, smoking, alcohol drinking status and hypertension, coronary heart disease (CHD) between AST/ALT ratio and DM. In the association between AST/ALT ratio and

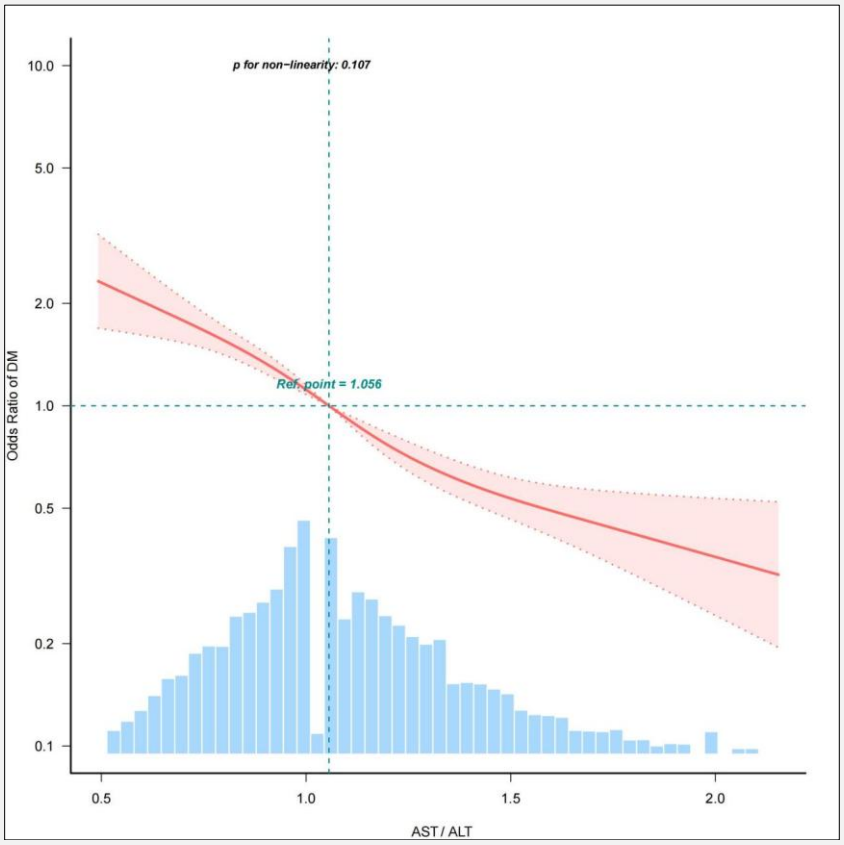

Figure 3. Adjusted fitting curve (Model 4) for the AST/ALT ratio in relation to diabetes mellitus (DM), showing a linear association (p for non-linearity = 0.107).

Additionally, an inverse correlation was observed between this ratio and DM risk.

diabetes mellitus (DM), a significant interaction was observed with race. Notably, non-Hispanic black exhibited a stronger inverse correlation between AST/ALT ratio and DM risk compared to other racial groups. Except for race, the relationship between AST/ALT ratio and DM was consistent and stable in all other stratified subgroups, ($p > 0.05$). (Figure 2). These results supported the notion that AST/ALT ratio might serve as a reliable biomarker for identifying individuals at risk of DM, irrespective of these factors. To assess the shape of this association, we conducted multivariable-adjusted analyses (Model 4) and fitted a curve, revealing a linear relationship (p for non-linearity = 0.107 (Figure 3).

DISCUSSION

The findings from this NHANES-based study suggested that the AST to ALT ratio may serve as a clinically useful biomarker for T2DM risk assessment. Our results align with and expand upon previous research linking liver enzyme levels to metabolic dysfunction, while also introducing new insights into the potential role of AST to ALT ratio in diabetes prediction.

Our analysis revealed that a higher AST to ALT ratio was associated with a lower risk of T2D, consistent with some prior studies suggesting that a low ALT relative to AST may reflect improved hepatic insulin sensitivity or reduced liver fat accumulation [16,17]. Since ALT is more liver-specific than AST, a decline in ALT (relative to AST) could indicate reduced hepatic steatosis, a known contributor to insulin resistance. To our knowledge, the study by Niu H et al. currently stands as the only published investigation examining the association between AST/ALT ratio and incident type 2 diabetes mellitus in PubMed-indexed literature. While their work identified a nonlinear relationship with an inflection point in a Japanese population, our analysis of the NHANES database revealed a significant linear inverse association in the US general population. This discrep-

ancy may be attributed to several important factors. First, substantial ethnic differences exist between the homogeneous Japanese cohort and the multi-ethnic NHANES population, particularly regarding body composition, insulin sensitivity, and liver enzyme metabolism patterns. Second, variations in study design - including differences in covariate adjustment, follow-up duration, and diagnostic criteria - may contribute to the distinct findings. Notably, our linearity test (p for nonlinearity = 0.107) provided no statistical evidence supporting a threshold effect in our dataset. The current lack of replicated studies in this area highlights the novelty of our findings and the need for further validation. Our study expands the existing evidence by demonstrating that: 1) this association may vary significantly across ethnic groups, and 2) the relationship may follow different patterns (linear vs. nonlinear) in different populations. These observations underscore the importance of population-specific analyses when evaluating potential biomarkers for diabetes risk prediction.

The potential mechanisms of the study are as follows: First, a lower ALT level (and thus a higher AST to ALT ratio) may correlate with better liver metabolic health, reducing gluconeogenesis and improving glycemic control. Second, since non-alcoholic fatty liver disease (NAFLD) is strongly linked to T2DM, a decreased ALT (relative to AST) might reflect less severe liver injury, thereby lowering diabetes risk. Third, some studies suggest that AST elevation (relative to ALT) in later stages of metabolic dysfunction could reflect mitochondrial stress, but our findings imply that a moderately elevated AST to ALT ratio may instead be protective.

Some prior studies have linked low AST to ALT ratio to worse metabolic outcomes, while others (like ours) suggest a protective effect of higher AST to ALT ratio. These discrepancies may stem from differences in study populations and adjustment for confounders in the cohort [18-20]. Previous studies have indicated a possible association between AST/ALT levels and type 2 diabetes mellitus (T2DM) [2,21,22]. However, these earlier investigations were limited by small sample sizes and insufficient adjustment for confounding factors, reducing the generalizability of their findings. The NHANES database provided an ideal platform to investigate the association between AST/ALT ratios and T2DM, allowing for a comprehensive assessment of their dose-risk relationship. Leveraging robust covariate adjustment and stratified analyses, our study strengthens the reliability of these findings. Notably, this is the first large-scale, multiethnic population-based analysis to establish a link between AST/ALT and T2DM among U.S. adults, thereby addressing a critical gap in the existing literature.

Nevertheless, several limitations should be considered when interpreting our findings. First, the cross-sectional nature of the study design prevents us from establishing causal relationships between AST/ALT and T2DM, highlighting the need for future prospective or longitudinal studies to confirm these associations. Second, the reliance on self-reported diabetes status may have introduced recall bias or diagnostic misclassification. Lastly, since our analysis focused exclusively on the U.S. adult population, the generalizability of the results to other ethnic groups or geographic regions remains uncertain.

CONCLUSION

Our study supports the potential utility of AST/ALT ratio as an easily accessible biomarker for T2DM risk, possibly reflecting underlying liver metabolism alterations. However, given the complexity of liver enzyme interactions with metabolic health, further validation is warranted before clinical implementation.

Declaration of Interests:
The authors declare no competing interests.

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