Association between blood methylmalonic acid and chronic kidney disease in the general US population: insights from multi-cycle National Health and Nutrition Examination Survey (NHANES)

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Background: Chronic kidney disease (CKD) is significantly influenced by mitochondrial dysfunction (MD). Previous research suggests that methylmalonic acid (MMA) is involved in MD. Consequently, we aimed to investigate associations between blood MMA level and the prevalence of CKD as well as mortality in patients with CKD.

Methods: The study included 23,587 individuals from National Health and Nutrition Examination Survey (NHANES). The NHANES datasets from 1999–2004 and 2011–2014 were utilized as separate primary and validation subsets. There were 3,554 patients with CKD. The association of blood MMA level with the prevalence of CKD was investigated using weighted logistic regression. Meanwhile, we employed weighted Cox regression models to evaluate the association between blood MMA level and all-cause mortality in patients with CKD.

Results: Blood MMA levels had a significant positive association with urinary albumin-to-creatinine ratio (β=45.29, P=0.01) and negative association with estimated glomerular filtration rate (β=−15.27, P<0.001) in CKD patients. Blood MMA level exhibited a significant increase in participants with CKD compared with those without CKD (7.60±0.86 *vs.* 7.03±0.62, P<0.001). The level of blood MMA was significantly associated with the prevalence of CKD [odds ratio (OR): 1.32, 95% confidence interval (CI): 1.05–1.64, P=0.01]. In addition, blood MMA level was significantly associated with all-cause mortality in CKD participants [hazard ratio (HR): 1.26, 95% CI: 1.11–1.43, P<0.001] after adjusting for other potential predictors.

Conclusions: Increased blood MMA levels were associated with more severe kidney impairment and increased risk of both the prevalence of CKD and mortality in participants with CKD.

Keywords: Methylmalonic acid (MMA); chronic kidney disease (CKD); mortality, mitochondrial dysfunction (MD)

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Introduction

Chronic kidney disease (CKD) is a widespread public health concern worldwide, resulting in end-stage kidney disease (ESKD). Based on the 2021 report by the Centers for Disease Control and Prevention (CDC), CKD affects over 1 in 7 American adults, with approximately 37 million individuals (1). Comprehending potential factors associated with CKD prevalence and outcome is important and might be helpful for devising efficacious therapeutic approaches.

Mitochondrial dysfunction (MD) is a central factor in CKD (2-5). The intermediate metabolite methylmalonic acid (MMA), produced through fatty acids and amino acids breakdown, enters the Krebs cycle when converted into succinic acid in normal circumstances (6). MMA was revealed to be involved in MD and oxidative stress (OS). An abnormal MMA accumulation occurs due to a deficiency of coenzyme active vitamin B12 (VitB12) or deactivation of mitochondrial methyl malonyl-CoA mutase (MUT) (7). Excessive accumulation of MMA could disrupt the electron transport chain, further impairing mitochondrial energy metabolism and producing reactive oxygen species (ROS) (8,9). Several studies found that the blood MMA level could be a useful marker for predicting poor outcomes of chronic diseases, including diabetes and cancer, associated with MD or OS (10,11). Moreover, the level of blood MMA was found to be an independent factor associated with all-cause mortality in the general population. This association was more prominent among patients with impaired kidney function (12,13). However, few investigations have concentrated on the associations between blood MMA levels and kidney impairment, the prevalence of CKD, as well as outcomes in CKD patients.

Herein, we explored the associations between blood MMA levels and kidney impairment, the risk of CKD prevalence, as well as all-cause mortality in CKD patients, utilizing the data from the National Health and Nutrition Examination Survey (NHANES), a comprehensive and long-term epidemiological survey conducted in the United States. We present this article in accordance with the STROBE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1930/rc).

Highlight box

Key findings

- Increased blood methylmalonic acid (MMA) levels were associated with more severe kidney impairment and increased risk of both chronic kidney disease (CKD) prevalence and mortality in participants with CKD.

What is known and what is new?

- Comprehending potential factors associated with the prevalence and outcomes of CKD is important and may be helpful in devising effective therapeutic approaches. MMA is involved in mitochondrial dysfunction (MD), which is a central factor in CKD.
- This study demonstrates that increased blood MMA levels were significantly associated with more severe kidney impairment and increased risk of both CKD prevalence and mortality in participants with CKD, utilizing the data from the National Health and Nutrition Examination Survey.

What is the implication, and what should change now?

- This study indicates that blood MMA, serving as a surrogate biomarker of MD, is independently and robustly associated with increased CKD prevalence and mortality in participants with CKD.
- The current study provides important clinical value for disease prevention and prediction of disease prognosis.

Methods

Study design and participants

The NHANES study is a comprehensive investigation of the health condition of non-institutionalized civilians across all age groups, employing a national, stratified, and multistage probability methodology. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All data were downloaded at https://www.cdc.gov/nchs/nhanes/. The present investigation incorporated 51,057 subjects who participated in five 2-year NHANES cycles (1999–2000, 2001–2002, 2003–2004, 2011–2012, and 2013–2014). We excluded 22,019 participants below the age of 18 years, 3,907 participants who had incomplete blood MMA data, 231 individuals with missing estimated glomerular filtration rate (eGFR), 394 participants without urinary albumin-to-creatinine ratio (uACR), 889 individuals who were pregnant, and 30 participants who lacked follow-up for mortality status. The study eventually enrolled a total of 23,587 participants. For primary analyses, a subset comprising the initial three cycles of NHANES 1999–2004 was utilized (n=13,530). The remaining two cycles (n=10,057) were used for validation. *Figure 1* illustrates the specifics of the recruitment process.

Data collection and definition

All data were collected from demographic surveys, physical examinations, and laboratory data in NHANES. Self-

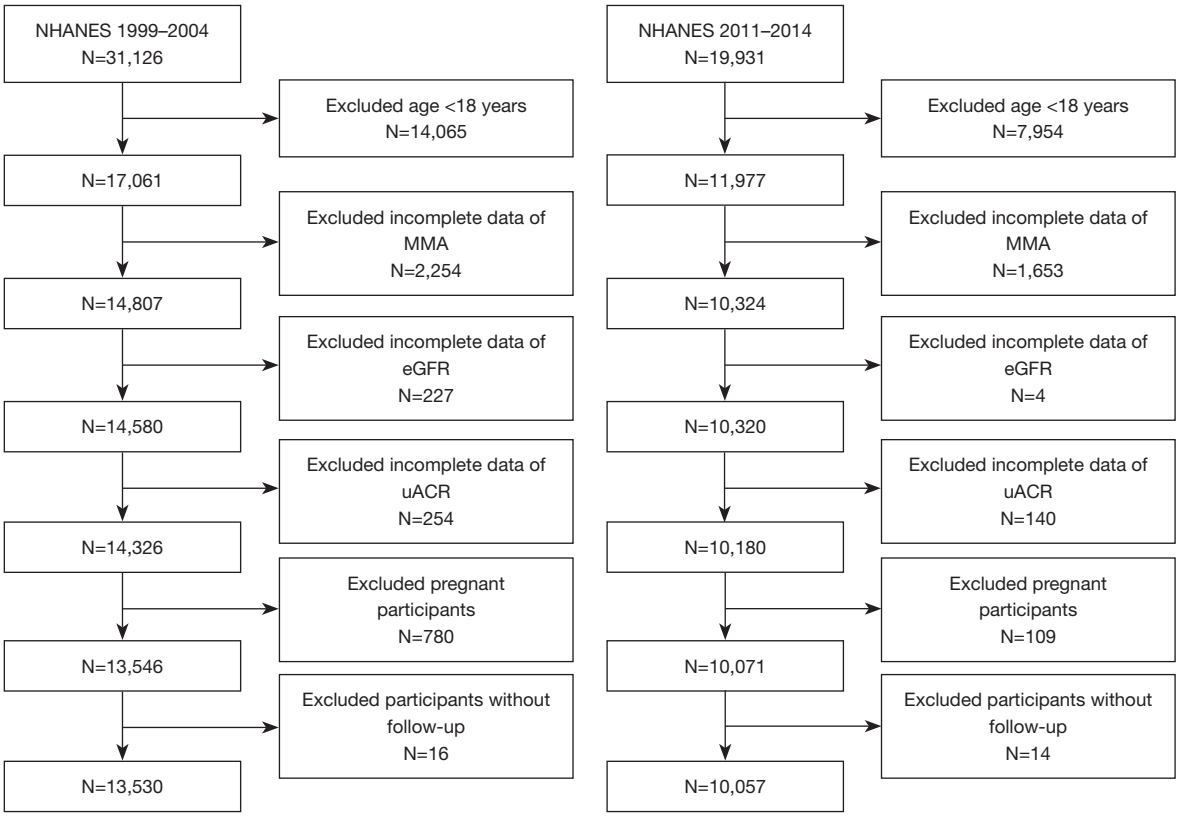

Figure 1 The detail for recruitment. NHANES, National Health and Nutrition Examination Survey; MMA, methylmalonic acid; eGFR, estimated glomerular filtration rate; uACR, urinary albumin-to-creatinine ratio.

administered questionnaires were utilized for obtaining general data including age, gender, race/ethnicity, and smoking status. The categorization of smoking status was never, ever, and current. Individuals who have smoked a minimum of 100 cigarettes in their whole life but have since ceased smoking were classified as "ever smokers".

Physical examinations were performed in mobile examination centers (MEC). The study categorized the participants based on their body mass index (BMI) categories into normal weight (BMI <25 kg/m^2) and overweight/obesity (BMI ≥25 kg/m^2). Average systolic blood pressure (SBP) or diastolic blood pressure (DBP) was calculated as the mean of eligible three readings. Hypertension was characterized by SBP ≥140 mmHg and/or DBP ≥90 mmHg, as determined through multiple examinations or a documented history of hypertension (14).

The laboratory measurements, including uACR, blood MMA level, serum creatinine (Scr), blood urea nitrogen (BUN), serum uric acid (UA), serum VitB12, triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), fasting blood glucose (FBG), and hemoglobin A1c (HbA1c) were conducted across all cycles. However, the measurements of C-reactive protein (CRP) and homocysteine were only available for the NHANES 1999–2004 cycle. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) was utilized for the eGFR calculation (15). In accordance with NHANES reports, cardiovascular disease (CVD) was characterized as a self-reported medical history of coronary heart disease, heart failure, or stroke. The definition of diabetes in this study was based on self-reported diabetes history and/or FBG ≥7.0 mmol/L and/or self-reported utilization of anti-diabetic drugs (16). The diagnostic criteria for hyperlipidemia were established as follows: TC ≥240 mg/dL, TG ≥200 mg/dL, LDL-C ≥160 mg/dL, or HDL-C <40 mg/dL (17). CKD was characterized by either albuminuria, lower eGFR, or both (18). The criterion for albuminuria was established as uACR ≥30 mg/g while defining reduced eGFR as an eGFR ≤60 mL/min/1.73 m^2.

The participants with an age of 18 years or above

in NHANES were associated with the National Death Index (NDI) through a distinctive sequence number until December 31, 2015 (19). Our study outcome was all-cause mortality.

Blood MMA measurement

Measuring blood MMA levels were performed in either plasma or serum, with a preference for plasma, in NHANES. The previous study validated that the reference range for MMA concentrations in both serum and plasma is consistent and that the coefficient of variation is comparable (20). The level of blood MMA was subjected to analysis through gas chromatograph/mass spectrometer (GC/MS, NHANES 1999–2004) or isotope-dilution high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, NHANES 2011–2014). The standard protocols for these methods are accessible on the NHANES website and were followed accordingly. Both methodologies exhibited a strong correlation and were devoid of any bias (21).

Statistical analyses

The data were reported as mean ± standard deviation (SD), or median and interquartile range (IQR) for continuous data, while as absolute frequencies and percentages for categorical data. Normalized expression data were log2-transformed. The comparisons of clinical features between the two groups were conducted through a weighted student's *t*-test for continuous data and a weighted chi-square test for categorical data. Weighted linear regression analysis with unstandardized regression coefficients (β) as effect measure was utilized for the correlation analysis between blood MMA levels and other clinical parameters, in which blood MMA levels acted as an independent variable, and eGFR, uACR, folate, serum VitB12, homocysteine, HDL-C, LDL-C, TG, TC, as well as CRP acted as dependent variables, respectively. The association between blood MMA levels and CKD prevalence was evaluated through the weighted logistic regression model. Specifically, model 1 followed an adjustment for age, gender, and race/ethnicity, model 2 followed further adjustment for BMI, smoking status, hypertension, diabetes mellitus (DM) history, and CVD history, and model 3 was further adjusted for eGFR, uACR, HbA1c, FBG, folate, serum VitB12, hyperlipidemia, and homocysteine. Results were reported as odds ratio (OR) with their 95% confidence interval (CI). Furthermore, stratification analyses were also applied for the association between blood MMA levels and CKD prevalence in subgroups by age (<45 and ≥45 years) (22), gender (female and male), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race), DM (yes or no), homocysteine (<10 and ≥10 μmol/L), and serum VitB12 (<400 and ≥400 pmol/L). Interaction analyses were incorporated to examine the heterogeneity of relations among various subgroups.

The study employed the Kaplan-Meier (K-M) analysis to estimate the survival of patients with CKD. The stratified log-rank test was utilized to evaluate any potential difference in survival rates. The current study employed weighted Cox proportional hazards model for estimating the association of blood MMA levels with all-cause mortality. Model 0 only comprised blood MMA levels, model 1 incorporated demographic variables (age, gender, race/ethnicity), and model 2 comprised variables from model 3 plus physical examination and medical history data (BMI, smoking status, hypertension, diabetes history, and CVD history). Finally, in model 4, laboratory variables were added. The relation robustness between MMA and mortality was additionally verified in NHANES 2011–2014, with the exception of homocysteine, by adjusting for the aforementioned potential confounders. Results were presented in terms of hazard ratio (HR) with 95% CI. Note that analyses were based on log2 transformed blood MMA value, the untransformed value was also used for data analyses. The statistical software package R (version 4.3.0) was employed for conducting the statistical analyses. P<0.05 indicated a significant difference.

Results

The level of blood MMA between participants with or without CKD

Of all participants enrolled in NHANES 1999–2004 (n=13,530), 1,761 (13.02%) were classified as CKD and 11,769 (86.98%) as non-CKD. A comparison of general characteristics between CKD and non-CKD participants is shown in *Table 1*. Participants with CKD were significantly older than those without CKD (64.45±17.42 *vs.* 43.43±15.95 years, P<0.001). Participants with CKD also showed significantly higher levels of SBP, folate, homocysteine, HbA1c, and CRP than those without CKD. Compared with participants without CKD, the prevalences

Table 1 General characteristics between CKD and non-CKD populations

Variables	CKD group (n=1,761)	Non-CKD group (n=11,769)	P value
Age (years)	64.45±17.42	43.43±15.95	<0.001
Gender (male/female)	43.38/56.62	49.87/50.13	<0.001
Race			0.004
Mexican American	5.00	7.57	
Other Hispanic	5.17	5.98	
Non-Hispanic White	72.87	71.73	
Non-Hispanic Black	11.59	10.15	
Other race	5.37	4.58	
BMI (<25/≥25 kg/m^2)	27.86/72.14	36.12/63.88	<0.001
Smoke (never/ever/current)	47.50/37.12/15.39	50.18/24.07/25.75	<0.001
SBP (mmHg)	137.97±25.01	121.18±17.26	<0.001
DBP (mmHg)	68.86±17.61	72.12±11.92	<0.001
Hypertension (yes/no)	73.60/26.40	31.06/68.94	<0.001
Diabetes (yes/no)	30.85/69.15	6.90/93.10	<0.001
CVD (yes/no)	30.95/69.05	6.31/93.69	<0.001
Hyperlipidemia (yes/no)	56.75/43.25	50.35/49.65	<0.001
FBG (mmol/L)	5.80 (5.24, 7.02)	5.28 (4.93, 5.69)	<0.001
HbA1c (%)	6.10±1.53	5.40±0.78	<0.001
eGFR (mL/min/1.73 m^2)	66.71±27.88	101.30±19.28	<0.001
Scr (μmol/L)	97.24 (79.56, 114.92)	70.72 (61.88, 79.60)	<0.001
uACR (mg/g)	31.86 (4.53, 75.13)	2.82 (1.92, 4.86)	<0.001
BUN (mmol/L)	6.10 (4.64, 8.20)	4.30 (3.57, 5.40)	<0.001
Folate (nmol/L)	32.40 (22.00, 50.50)	27.40 (19.70, 38.50)	<0.001
VitB12 (pmol/L)	351.29 (256.82, 466.42)	336.53 (258.30, 440.59)	0.06
Homocysteine (μmol/L)	10.85 (8.49, 13.79)	7.80 (6.51, 9.38)	<0.001
CRP (mg/dL)	0.29 (0.13, 0.65)	0.19 (0.07, 0.42)	<0.001
TC (mmol/L)	5.22 (4.47, 5.97)	5.09 (4.47, 5.84)	0.004
TG (mmol/L)	1.64 (1.20, 2.45)	1.29 (0.89, 1.89)	<0.001
HDL-C (mmol/L)	1.24 (1.03, 1.53)	1.28 (1.06, 1.58)	0.16
LDL-C (mmol/L)	3.03 (2.48, 3.62)	3.05 (2.46, 3.67)	0.23
Blood MMA (log2)	7.60±0.86	7.03±0.62	<0.001

Data are presented as mean ± standard deviation, median (interquartile range), or percentage. CKD, chronic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; uACR, urine albumin-to-creatinine ratio; BUN, blood urea nitrogen; VitB12, vitamin B12; CRP, C-reactive protein; TC, total cholesterol; TG, total triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MMA, methylmalonic acid.

Table 2 Associations of CKD with blood MMA in NHANES 1999–2004

Variables	OR	95% CI	P value
M0	2.91	2.67–3.16	<0.001
M1	2.04	1.85–2.25	<0.001
M2	1.95	1.78–2.13	<0.001
M3	1.32	1.05–1.64	0.01

M0 (n=13,530): blood MMA; M1 (n=13,530): additionally adjusted for age, gender and race/ethnicity; M2 (n=11,541): additionally adjusted for smoke status, BMI, hypertension, diabetes, CVD; M3 (n=5,661): additionally adjusted for eGFR, uACR, HbA1c, FBG, folate, serum VitB12, hyperlipidemia, homocysteine. CKD, chronic kidney disease; MMA, methylmalonic acid; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; CI, confidence interval; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; uACR, urine albumin-to-creatinine ratio; HbA1c, hemoglobin A1c; FBG, fasting blood glucose; VitB12, vitamin B12.

of CVD, hypertension, hyperlipidemia, and diabetes were significantly higher in those with CKD. Moreover, blood MMA levels were significantly higher in participants with CKD than those without CKD (7.60±0.86 *vs.* 7.03±0.62, P<0.001). Even after adjusting for eGFR and serum VitB12, the level of blood MMA in participants with CKD was still significantly higher than those without CKD [mean (95% CI): 7.29 (7.25–7.33) *vs.* 7.04 (7.03–7.05), P<0.001]. These results were validated in the NHANES 2011–2014 validation cohort (Table S1).

Associations of blood MMA levels with clinical parameters

Next, the associations between the blood MMA levels and clinical parameters in patients with CKD were evaluated using weighted linear regression. In NHANES 1999–2004, it was found that blood MMA had a significant positive association with uACR (β=45.29, P=0.01) and homocysteine (β=4.23, P<0.001). Conversely, blood MMA had a significant negative association with eGFR (β=−15.27, P<0.001) and serum VitB12 (β=−78.92, P=0.003). Nevertheless, no significant association was detected between blood MMA level and HDL-C, LDL-C, TG, TC, FBG, HbA1c, or CRP. Furthermore, the significant association between MMA and kidney impairment was further validated in NHANES 2011–2014. We found that blood MMA level was positively correlated with uACR (β=89.96, P<0.001) and inversely correlated with eGFR (β=−20.06, P<0.001) in the validation cohort.

Association between the level of blood MMA and CKD prevalence

With the use of weighted logistic regression, higher blood MMA levels were significantly associated with a higher CKD risk (OR: 2.91, 95% CI: 2.67–3.16, P<0.001). The results maintained statistical significance following the adjustment for age, gender, and race/ethnicity (OR: 2.04, 95% CI: 1.85–2.25, P<0.001). The association also maintained statistical significance following the adjustment for potential risk factors, including age, gender, race/ethnicity, BMI, smoking status, hypertension, diabetes history, CVD history, eGFR, uACR, HbA1c, FBG, folate, serum VitB12, hyperlipidemia and homocysteine (OR: 1.32, 95% CI: 1.05–1.64, P=0.01, *Table 2*). The aforementioned findings were verified in the validation cohort of NHANES 2011–2014 (Table S2).

The results of the stratified and interaction analyses of the association between blood MMA level and CKD prevalence are presented in *Figure 2*. For the subgroup stratified by gender, diabetes, homocysteine and serum VitB12, blood MMA levels were found to be significantly associated with the prevalence of CKD in each subgroup (P<0.05). The stratified analyses demonstrated that blood MMA level was significantly associated with CKD in participants older than 45 years (adjusted OR: 1.83, 95% CI: 1.55–2.17, P<0.001). In Mexican American, non-Hispanic White and non-Hispanic Black, blood MMA levels were associated with a greater risk of CKD prevalence (adjusted OR: 1.81, 95% CI: 1.16–2.81, P=0.009; adjusted OR: 1.67, 95% CI: 1.36–2.05, P<0.001; adjusted OR: 2.21, 95% CI: 1.22–4.01, P=0.009, respectively). The interaction analyses revealed no interactive role in the association between the level of blood MMA and CKD prevalence.

Association of blood level of MMA with all-cause mortality in CKD patients

To investigate the association between blood level of MMA and all-cause mortality in patients with CKD, we gathered data on NHANES participants with CKD and linked them to NDI for follow-up of their survival. During the 1,761 person-years follow-up (median: 12.7 years), 1,219 patients died (all-cause mortality). We found that a higher level of blood MMA at baseline exhibited a significant association with an elevated risk of all-cause mortality during

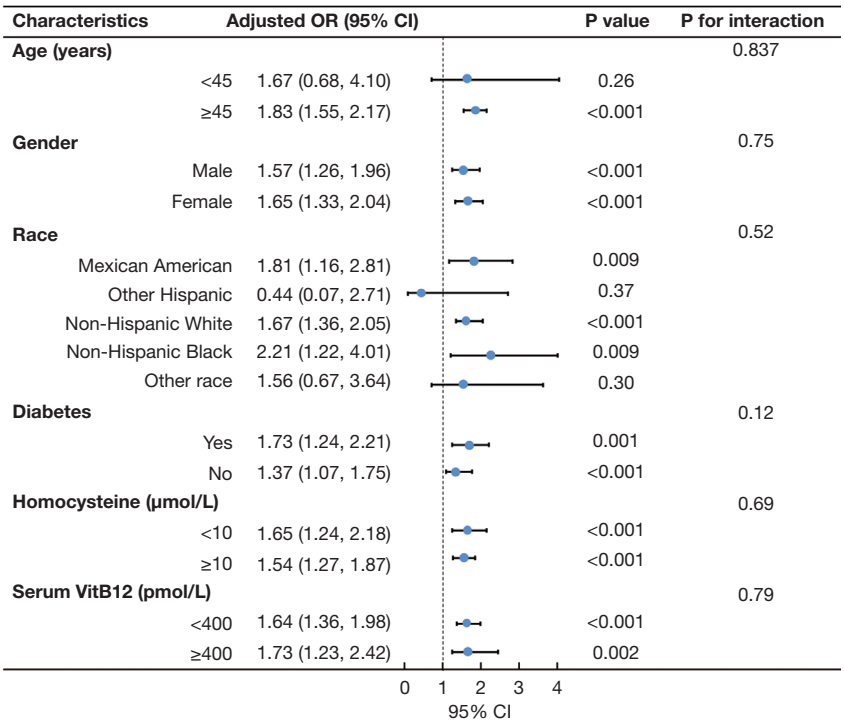

Figure 2 The associations between blood MMA and the prevalence of CKD in various subgroups. MMA, methylmalonic acid; CKD, chronic kidney disease; OR, odds ratio; CI, confidence interval; VitB12, vitamin B12.

the follow-up (*Figure 3A*). After adjustment for all relevant confounders, a higher level of blood MMA was significantly associated with an increased risk of all-cause mortality (log-rank test, P<0.001). Weighted univariable regression analysis also identified the increased blood MMA level as a predictor for all-cause mortality in patients with CKD (HR: 1.54, 95% CI: 1.44–1.64, P<0.001). Furthermore, multivariable Cox regression analysis indicated that the level of blood MMA remained an independent predictor for all-cause mortality, even after the adjustment for age, gender, race/ethnicity, BMI, smoking status, hypertension, diabetes history, CVD history, eGFR, uACR, HbA1c, FBG, hyperlipidemia, folate, serum VitB12 and homocysteine (HR: 1.26, 95% CI: 1.11–1.43, P<0.001; *Table 3*).

The association between the level of blood MMA and all-cause mortality in CKD patients was verified in 1,793 participants of NHANES 2011–2014. Throughout the median 6.4-year follow-up, 460 patients died. Similarly, the K-M curve and multivariable Cox regression analyses suggested a significant association between blood MMA levels and the risk of all-cause mortality in the validation cohort (HR: 1.35, 95% CI: 1.14–1.60, P<0.001, shown in *Figure 3B* and Table S3).

Discussion

Elevated circulating MMA levels have been reported in several diseases in adults, such as coronary heart disease, kidney dysfunction, and diabetes (23-25). Moreover, emerging evidence suggested that elevated level of blood MMA may serve as a promising biomarker for predicting adverse outcomes in chronic diseases (12,13,26). As we know, MD is regarded as a contributing factor to CKD progression (27). However, whether the level of blood MMA is associated with the risk of CKD and mortality of CKD participants is of interest for investigation.

Herein, a significant elevation was detected in the level of blood MMA among the participants with CKD compared with those without CKD. Meanwhile, we observed that the blood MMA levels were significantly correlated with kidney impairment in patients with CKD. MMA is one of the biomarkers involved in VitB12 metabolism, alongside folate and homocysteine (28). It was reported significant differences in serum VitB12, folate, and homocysteine levels between CKD and non-CKD populations (29), which was consistent with our findings. However, comparing with serum VitB12, folate and homocysteine, blood level

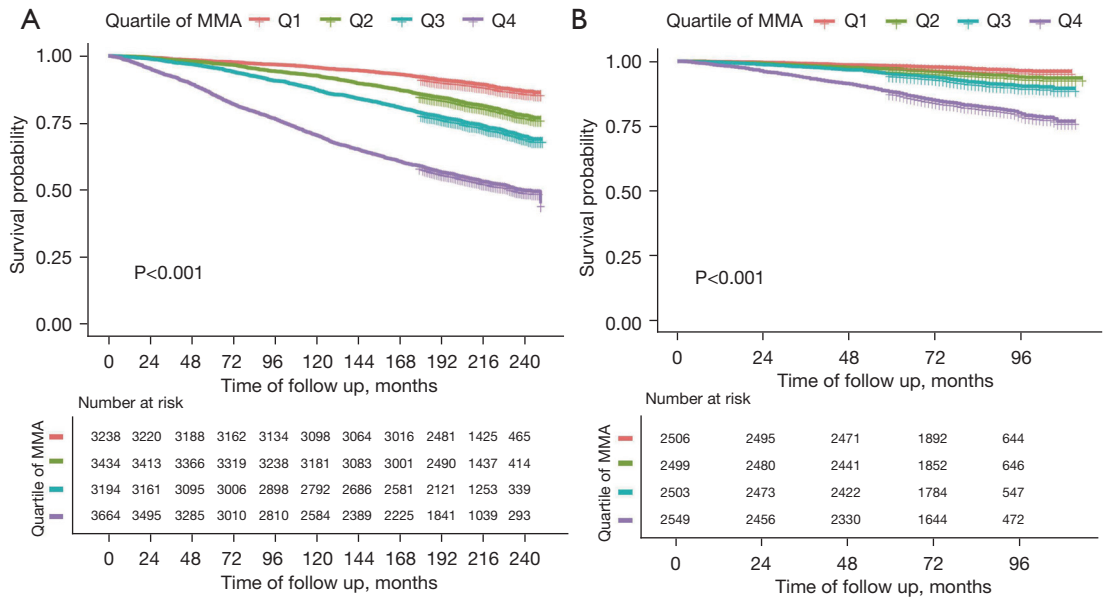

Figure 3 Kaplan-Meier analyses for all-cause mortality by baseline blood MMA strata. (A) Participants with CKD in NHANES 1999–2004; (B) participants with CKD in NHANES 2011–2014. MMA, methylmalonic acid; CKD, chronic kidney disease; NHANES, National Health and Nutrition Examination Survey.

Table 3 Weighted Cox's proportional hazard models for all-cause mortality in CKD patients of NHANES 1999–2004

Variables	HR	95% CI	P value
M0	1.54	1.44–1.64	<0.001
M1	1.31	1.21–1.41	<0.001
M2	1.29	1.21–1.38	<0.001
M3	1.26	1.11–1.43	<0.001

M0 (n=1,761): blood MMA; M1 (n=1,761): additionally adjusted for age, gender and race/ethnicity; M2 (n=1,576): additionally adjusted for smoke status, BMI, hypertension, diabetes and CVD; M3 (n=764): additionally adjusted for eGFR, uACR, HbA1c, FBG, folate, serum VitB12, hyperlipidemia and homocysteine. CKD, chronic kidney disease; NHANES, National Health and Nutrition Examination Survey; HR, hazard ratio; CI, confidence interval; MMA, methylmalonic acid; BMI, body mass index; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; uACR, urine albumin-to-creatinine ratio; HbA1c, hemoglobin A1c; FBG, fasting blood glucose; VitB12, vitamin B12.

of MMA had a more sensitive and specific performance in reflecting VitB12 homeostasis (30). The disruption of VitB12 homeostasis directly leads to adverse effects of cardiovascular risk and CKD progression (31,32). In addition, the level of blood MMA was found to have a significant association with all-cause mortality in the general population (12). In the present study, we extended previous findings and, for the first time, found that higher MMA levels exhibited a significant association with an elevated risk of CKD prevalence and all-cause mortality in patients with CKD. The associations between blood MMA and the prevalence of CKD patients in the stratified analyses were consistent with that in the multivariable logistic regression analysis.

Although blood level of MMA is primarily recognized as a functional marker of VitB12 deficiency and is influenced by renal function (24), there is increasing evidence suggesting that only a portion of the variation in MMA levels could be explained by VitB12 and eGFR (13,33,34). Consistent with prior studies, our findings demonstrated that even after adjusting for eGFR and VitB12, patients with CKD still exhibited significantly higher blood MMA level compared with the non-CKD group. Increasing evidence has supported that MMA is not only a metabolite indicating VitB12 deficiency but also a marker for MD and OS (35,36). A recent study demonstrated that proximal tubular MD is a key pathogenic mechanism behind MMA-related kidney disorders, and antioxidants can attenuate the severity of nephropathy (37). However, the toxicity of MMA in MD was revealed to be mainly based on congenital methylmalonic acidemia (8,38). Mitochondrial damage

could exacerbate kidney damage, leading to a vicious cycle in CKD progression (39,40). We assumed that MMA may be involved in the pathogenesis of CKD.

This study has several strengths. Regarding current knowledge, this study presents the initial investigation documenting the associations between circulating MMA and CKD prevalence. Using a nationally representative sample improves the extent to which our findings can be generalized. Nonetheless, there were no supplementary data beyond mortality for follow-up purposes in NHANES analysis. Subsequent investigations should document repeated measurements of eGFR and questionnaires to authenticate the reported associations. There are some limitations to our study. The current study is observational, and additional research is required to ascertain whether the relationship between MMA and kidney progression in patients with CKD is causal.

Conclusions

Blood MMA level was positively associated with CKD prevalence. Moreover, blood MMA level was an independent prognostic factor in the mortality of patients with CKD.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-23-1930/rc

Peer Review File: Available at https://atm.amegroups.com/article/view/10.21037/atm-23-1930/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1930/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Basic characteristics of participants with or without CKD in NHANES 2011–2014

Variables	CKD group (n=1,793)	Non-CKD group (n=8,264)	P value
Age (years)	59.70±17.67	45.13±15.85	<0.001
Gender (male/female)	42.64/57.36	50.03/49.97	<0.001
Race			0.004
Mexican American	7.30	8.83	
Other Hispanic	5.09	6.17	
Non-Hispanic White	68.09	66.68	
Non-Hispanic Black	12.75	10.38	
Other race	6.76	7.93	
BMI (<25/≥25 kg/m^2)	26.07/73.93	30.74/69.26	<0.001
Smoke (never/ever/current)	49.30/33.14/17.56	57.54/22.41/20.05	<0.001
SBP (mmHg)	130.86±21.53	120.05±15.14	<0.001
DBP (mmHg)	68.92±15.16	70.94±11.15	<0.001
Hypertension (yes/no)	66.24/33.76	33.11/66.89	<0.001
Diabetes (yes/no)	31.80/68.20	9.33/90.67	<0.001
CVD (yes/no)	24.29/75.71	5.67/94.33	<0.001
Hyperlipidemia (yes/no)	57.92/42.08	57.46/42.54	0.74
HbA1c (%)	6.19±1.47	5.53±0.79	<0.001
eGFR (mL/min/1.73 m^2)	72.42±29.27	97.38±17.90	<0.001
Scr (μmol/L)	90.17 (69.84, 113.15)	74.26 (63.65, 85.75)	<0.001
uACR (mg/g)	40.18 (13.04, 90.00)	6.19 (4.28, 9.64)	<0.001
BUN (mmol/L)	5.71 (3.93, 7.50)	4.28 (3.57, 5.36)	<0.001
Folate (nmol/L)	42.80 (28.90, 66.10)	39.00 (26.80, 55.40)	<0.001
VitB12 (pmol/L)	391.90 (275.30, 554.20)	377.10 (283.40, 510.00)	0.003
TC (mmol/L)	4.78 (4.14, 5.69)	4.91 (4.24, 5.61)	0.63
TG (mmol/L)	1.31 (0.90, 1.91)	1.12 (0.77, 1.66)	0.001
HDL-C (mmol/L)	1.27 (1.03, 1.58)	1.32 (1.09, 1.58)	0.105
LDL-C (mmol/L)	2.66 (2.10, 3.39)	2.90 (2.33, 3.49)	0.001
Blood MMA (log2)	7.58±0.77	7.16±0.61	<0.001

Data are presented as mean ± standard deviation, median (interquartile range), or percentage. BMI, body mass index; BUN, blood urea nitrogen; CKD, chronic kidney disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MMA, methylmalonic acid; SBP, systolic blood pressure; NHANES, National Health and Nutrition Examination Survey; Scr, serum creatinine; TC, total cholesterol; TG, total triglyceride; uACR, urine albumin-to-creatinine ratio; VitB12, vitamin B12.

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Table S2 Associations of CKD with blood MMA in NHANES 2011–2014

Variables	OR	95% CI	P value
M0	2.43	2.18–2.71	<0.001
M1	1.86	1.67–2.08	<0.001
M2	1.78	1.59–1.98	<0.001
M3	1.33	1.04–1.68	0.023

M0 (n=10,057): blood MMA; M1 (n=10,057): additionally adjusted for age, gender and race/ethnicity; M2 (n=9,818): additionally adjusted for smoke status, BMI, hypertension, diabetes, CVD; M3 (n=4,696): additionally adjusted for eGFR, uACR, HbA1c, FBG, folate, serum VitB12 and hyperlipidemia. BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; MMA, methylmalonic acid; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio; uACR, urine albumin-to-creatinine ratio; VitB12, vitamin B12.

Table S3 Weighted Cox's proportional hazard models for all-cause mortality in CKD patients of NHANES 2011–2014

Variables	HR	95% CI	P value
M0	1.77	1.59–1.97	<0.001
M1	1.41	1.28–1.55	<0.001
M2	1.36	1.22–1.52	<0.001
M3	1.35	1.14–1.60	<0.001

M0 (n=1,793): blood MMA; M1 (n=1,793): additionally adjusted for age, gender and race/ethnicity; M2 (n=1,742): additionally adjusted for smoke status, BMI, hypertension, diabetes and CVD; M3 (n=812): additionally adjusted for eGFR, uACR, HbA1c, FBG, folate, serum VitB12 and hyperlipidemia. BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; MMA, methylmalonic acid; NHANES, National Health and Nutrition Examination Survey; HR, hazard ratio; uACR, urine albumin-to-creatinine ratio; VitB12, vitamin B12.

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