Peer Review File

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Reviewer A

Wu et al. submitted their comprehensive work on "Association between blood methylmalonic acid (MMA) and chronic kidney disease (CKD) in the general US population: Insights from multi-cycle NHANES" and examined the association between MMA and CKD in a big cohort. The authors suggest that MMA levels in blood are in direct correlation with CKD occurrence in the general population. The manuscript is an easy and comprehensive read and presents the arguements with good data and tables. At this time I do not have any major correction suggestions and find the manuscript eligible for publishing as it is.

Reply: Thank you for your comments. We appreciate your positive feedback.

Reviewer B

Wu et al studied association of methylmalonic acid (MMA) as a marker of mitochondrial dysfunction (MD) and severity of chronic kidney disease (CKD) in multi-cycle NHANES data in 1999-2004 and 2011-2014, conducted in the US. They used weighted logistic regression as well as cox-regression to assess the association of MMA and mortality in individuals with CKD. They reported significant association of MMA levels and urinary albumin-to-creatinine ratio (β = 45.29, P = 0.019) and negative association with estimated glomerular filtration rate (β = -15.27, P < 0.001) in CKD patients. They reported higher Blood MMA level in participants with CKD compared with those without CKD (7.60±0.86 vs. 7.03±0.62, P<0.001). The y also stated that the level of blood MMA was significantly associated with CKD occurrence (OR [95% CI]: 1.32 [1.05 to 1.64], P=0.017), as well as with all-cause mortality in CKD participants (HR [95%CI]: 1.26 [1.11 to 1.43], P<0.001) after they adjusted for other potential predictors. They concluded that "increased blood MMA levels were associated with more severe kidney impairment and increased risk of both CKD occurrence and

mortality in participants with CKD."

There are several concerns:

1- The authors serval times mentioned that they assessed the occurrence of CKD and levels of MMA.

The NHANES data is cross-sectional not longitudinal and any information on CKD is prevalence not incidence or occurrence. Therefore, the occurrence of CKD or risk of CKD need to be changed to prevalence of CKD.

Reply 1: Thank you very much for the reminder. According to your recommendation, the occurrence of CKD or risk of CKD has been changed to the prevalence of CKD in the revised manuscript.

Changes in the text: We have modified our text as advised (see Page 3, line 28, 34, 42; Page 4, line 47; Page 5, line 49; Page 6, line 54, 68, 70; Page 10, line 139, 145; Page 12, line 190, 200; Page 13, line 208; Page 15, line 249, 250, 265; Page 16, line 274; Page 19, line 390).

2- Methylmalonic acid increases with vitamin B12 deficiency. Although Vit B12 deficiency has been reported with CKD, whether mitochondrial disfunction is underlying factor of increasing MMA or Vit B12 level is an issue. Although with adjustment for Vit B12 levels, the association of MMA and CKD remained significant, in the stratified analyses those with Vitamin B12 less than 400 unit the association was not significant. One could interpret the level of MMA is due to B12 levels and therefore, malfunction of methylmalonyl CoA mutase.

Reply 2: Thank you for your comments. According to your recommendation, we did not adjust for eGFR in stratified analyses, and recalculated stratified and interaction analyses. The updated results are shown in Figure 2 in the revised manuscript. For the subgroup stratified by vitamin B12, we found that the blood MMA levels were significantly associated with the prevalence of CKD in both serum vitamin B12 levels ≥400 and <400pmol/L ($P < 0.05$).

Changes in the text: We have modified our text as advised (see Page 12, line 200-202;

Figure 2).

3- Similarly, in those with homocysteine less than 10 unit there was no association between CKD and MMA.

From the stratified analyses which is a much stronger approach for confounders, it seems that MMA is due to variability in B12 levels. I suggest repeating the analyses for individuals with B12 level >400 and homocysteine <10 in those older than 65 years old and re-assess the associations. I suggest not to adjust for eGFR as eGFR is already incorporated in the definition of CKD. If there is any association for MMA and CKD, eGFR or ACR in those older than 65 years old but normal B12 >400 and homocysteine <10, that supports the discussion statement "Increasing evidence has supported that MMA is not only a metabolite indicating VitB12 deficiency but also a marker for mitochondrial dysfunction and oxidative stress", otherwise it is hard to accept mitochondrial dysfunction as explanation of MMA and CKD.

One could argue that B12 levels and CKD are associated through MMA level and that MMA is mediator in the pathway. However, relating MMA to mitochondrial function with current data is rather speculation.

Reply 3: Thank you for your comments. According to your recommendation, we did not adjust for eGFR and recalculated stratified analyses. We found that the blood MMA level was significantly associated with the prevalence of CKD in both homocysteine <10μmol/L and >10μmol/L groups.

In addition, we conducted stratified analyses for participants older than 65 years old. We found that serum vitamin B12 and homocysteine were significantly associated with the prevalence of CKD in each subgroup in those older than 65 years old ($P < 0.05$), as shown in the Table below. Therefore, as you mentioned, these results support our viewpoint that "increasing evidence has supported that MMA is not only a metabolite indicating VitB12 deficiency but also a marker for mitochondrial dysfunction and oxidative stress."

Characteristics	OR(95% CI)	*P* value	*P* for

				interaction
Serum vitamin B12				
<400	1.50	(1.17, 1.93)	0.002	0.383
≥400	1.92	(1.16, 3.17)	0.011	
Homocysteine				
<10	1.53	(1.02, 2.30)	0.041	0.590
≥10	1.74	(1.35, 2.24)	<0.001	

Changes in the text: We have modified our text as advised (see Page 12, line 200-202; Page 13, line 204-207; Figure 2).

4- It is not clear why cut point 45 years was considered for age. In stratified analyses I suggest avoiding over-adjustment. The main risk factor sof CKD are age, diabetes, systolic blood pressure and inflammation. In the above stratified analyses only include these factors in the model. Moreover, assess the link between systolic blood pressure and MMA and DM. If they are associated, then MMA could be a common risk factor between all of them. Therefore, in that case, do not adjust for SBP or diabetes, but for age and ethnicity, and income.

Hope these suggestions can help to disentangle the associations.

Reply 4: Thank you for your comments. The selection of the cut point at 45 years was based on age stratification for CKD [*Magdalena Madero. KDIGO 2023, ERA 2023*] and is consistent with similar studies in the field [*Qian D, et al, Front Med (Lausanne). 2022; Thang OH, et al, Nephron Extra. 2012*]. Relevant literature supporting this choice has been added to the manuscript.

We calculated the correlation between blood MMA levels and systolic blood pressure as well as diabetes. The results showed a significant correlation between blood MMA levels and systolic blood pressure (P=0.009), but no significant correlation between blood MMA levels and diabetes (P=0.507). According to your suggestion, we have only included main risk factors of CKD in the stratified analyses and re-analyzed the results. The corresponding results have been added in Figure 2 in the revised manuscript.

Changes in the text: We have added the relevant references accordingly (see Ref. 22). In addition, we have modified our text as advised (see Figure 2).