## The association between vitamin C deficiency and metabolic syndrome among patients who developed kidney disease.

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#### Abstract

(i) (ii)

**Introduction**: Elevated albuminuria is an important outcome of diabetic complications and metabolic syndrome (MetS), the complex metabolic abnormalities manifested as glucose intolerance, hypertension, and dyslipidemia and enlarged waist circumference. These coexisting cardiometabolic abnormalities are also associated with oxidative stress and low grade inflammation. Vitamin C (vitC) has long been recognized as hydrophilic antioxidant and blocker of oxidative stress which also has a protective role of MetS and kidney disease. **Objective**: The aim of this study was to identify the predictive effect of vitC deficiency on kidney disease among patients who developed metabolic syndrome.

**Method and procedures**: To meet this objective, the National Health and Nutrition Examination Survey (NHANES) 2007-2008 data were used. NHANES represents the civilian, non-institutionalized US population data implemented for continuous system of monitoring health, nutritional status and well-being in the US. The predictive power of MetS on AU and vitC deficiency (vitC < 0.25 mg/dl) as effect modifier was assessed with binary logistic regression. Age, gender, race and BMI were used as covariates.

**Results**: Among the respondents, 1.9% of the US population had risk of kidney damage, whereas 14.5 % of respondents had MetS. MetS was significantly positively correlated with AU (p < 0.01). Higher elevation of albuminuria creatinine ratio (ACR) was found in participants with VCD. The regression models developed in this study showed that the severity of kidney disease measured by ACR was increased among individuals who developed both VCD and MetS compared with individuals who developed MetS only (AOR=5.53; 95% CI: 2.73, 11.21). Increasing age and being African-American in race were independently associated with elevated AU.

**Conclusion**: Specific considerations tailored to metabolic syndrome, vitamin C and kidney diseases measured by ACR screening and monitoring mechanisms at the community level is critical to tackle further medical complications. Moreover, the role of VitC fortified foods on prevention of kidney diseases among patients with MetS warrant further investigation.

Key words: Metabolic syndrome, vitamin C deficiency, kidney disease, Albuminuria, USA

## Introduction

Kidney disease is nowadays becoming a critical public health problem by accounting as the ninth cause of death in the United States affecting about 20 million Americans [1]. In the US, approximately 6% of men and 9.7% of woman have albuminuria [2]. Albuminuria (AU), defined as the excretion of over 30 mg/g urinary albumin creatinine ratio, is known to be the most basic indicator of kidney impairment and is a tool for an early detection of diabetic nephropathy [3]. AU is an important extrapolative factor for the progress and complications of diabetes and the related adverse events in patients with type I and type II DM and blood pressure (BP)[4]. Though the mechanism is not fully understood, chronic kidney disease (CKD) is associated with cardiovascular disease (CVD) and mortality associated with diabetes [5, 6]. Recent clinical trials based on morning spot urine tests showed that albuminuria predicts mortality from cardiovascular, renal and all causes mortality better than glucose status and BP [7].

Glucose intolerance, hypertension, dyslipidemia and large waist circumference constitute the socalled metabolic syndrome (MetS[8]. These factors are all linked to an increased risk of insulin resistance, diabetes, kidney disease and cardiovascular disease [9, 10]. MetS is associated with elevated albuminuria [11], directly or indirectly through insulin resistance, increased proinflammatory cytokines and reduced anti-inflammatory cytokines [12]. Reduced hemodynamic dysfunction of insulin and bioavailability of nitric oxide affect the vasculature leading to endothelial dysfunction [13], and aggravated IR. IR may result from inflammation induced insulin resistance, namely inhibition of the PI3K-Akt pathway subsequently leading to reduced Akt phosphorylation and glucose uptake, glycogen or fat synthesis [13, 14].

Targeting oxidative stress and inflammation as a means of preventive or treatment options in conjunction with hypoglycemic agents may be beneficial. This is because of reactive oxygen species with oxidative stress leads to cellular death of the vital organs[15]. For this, ascorbic acid (vitC) has a powerful antioxidant scavenging free radicals such as hydroxyl radical and singleton oxygen[16] that may damage the renal tissue [15].

So that, the primary purpose of this study was to determine the predictive effect of MetS on AU and how this is provoked by the presence of vitC deficiency (VCD). We assumed that MetS predict AU and vitC deficiency may deteriorate the advancement of kidney disease. Age, gender, race and BMI were used as covariates to adjust for possible confounding.

#### **Methods and Procedures**

**Study Data:** NHANES 2007–2008 data were used to examine the association of metabolic syndrome and kidney disease assessed by urinary albumin excretion. NHANES data represent the civilian, non-institutionalized US population. Subject recruitment was based on a complex multistage, stratified sampling method. Screening visit, visit for health status interview and examinations including physical, body measurements, blood and urine collections, at a mobile examination center (MEC) [17].

**Laboratory Analyses:** Vitamin C (measured as serum ascorbic acid, an indicator of tissue stores)[18]. Laboratory analysis of urinary albumin followed the procedure of Chavers et al [19] using a non-competitive, double-antibody solid-phase fluorescent immunoassay. The first antibody to human albumin was covalently attached to derivatized polyacrylamide beads, this was reacted with urine specimen and the urine albumin-antigen complexes were further reacted with the second fluorescein-labeled antibody. The fluorescence level was determined within functional range of 0.5–20  $\mu$ g/mL of albumin. Data were corrected for creatinine determined from the same specimen. Creatinine, the waste product derived from creatine, is released into the plasma at a

relatively constant rate assuming that the amount of creatinine per unit of muscle mass is constant. Creatinine is also an indicator of impaired kidney function. Creatinine analysis is based on reaction rate measurements with picrate in an alkaline solution to form a red creatinine-picrate complex at two wavelengths 520 and 560 nm according to established protocols.

#### Variable Definitions

Metabolic syndrome (MetS) was defined according to the latest definition of ATP III from the American Heart Association (AHA)/National Heart, Lung, and Blood Institute (NHLBI) and the 2009 Joint Scientific Statement[8]. Data files were combined using SPSS; the variable MetS was created according to the standard definition that 3 out of 5 risk factors were used to determine metabolic syndrome (MetS) [20-22]: 1) Waist circumference (WC)  $\geq$  102 cm (males adults) and  $\geq$ 88 cm (female adults); 2) FG  $\geq$  100 mg/dl; 3) BP  $\geq$  130/85 mm Hg (either); 4) TG  $\geq$  150 mg/dl; and 5) HDL <40 mg/dl (male adults) and <50 mg/dl (female adults). WC was measured at the high point of the iliac crest at minimal respiration to the nearest 0.1 cm. Serum TG concentrations were determined with the enzymatic hydrolyzation to glycerol. HDL was determined by the lipoprotein precipitation method using heparin-manganese chloride. FBG was determined by standard enzymatic method. Average of all available BP measurements was used in this study. Variables were computed both in the form of continuous or categorical values. When categorized, conventional literature values were used. The following cut-off points were used for the different variables: Urinary albumin: creatinine ratio (ACR) above 30 mg/g was defined as albuminuria (AU). In this paper ACR was used to represent the continuous variable and AU was used to represent the categorical variable. In this study, Low serum vitamin C is defined as less than 0.25 mg/dl; unpublished data from the second National Health and Nutrition Examination Survey of the US population from 1976-1980.

#### Statistical methods

Data analyses were performed on the NHANES, 2003–2006 survey using SPSS (Statistical Analysis for Social Scientists version 24, IBM, Armonk, NY, USA) software. Descriptive statistics included mean comparisons, *t*-tests and ANOVA for continuous, normally distributed variables, and proportions and Chi-squared tests for ordinal and nominal variables. Binary logistic regression was fitted to assess the strength of association between the outcome variable AU and the predictor variable MetS. The association of ACR with vitC status was first determined. VCD was identified as effect modifier through statistical computations of binary logistic regression before and after adjusting for, without and with consideration of the interaction terms. Covariates

considered to be potential confounders included age groups, gender, BMI( Underweight, normal and overweight) and race/ethnicity stratified into three groups (White, African American, Others).

## Results

A total population of 10,348 from NHANES 2007-2008 was included in the analysis. The proportion of female participants was 50.9 % as compared to men (49.1%). The mean  $\pm$  with its corresponding standard error of age of participants was 28.06 $\pm$ 0.34 years for men (N=5,080) and 27.9  $\pm$  0.33 years for women (N=5,268). With regard to race distribution, 2,710 (26.2&) were African Americans; 3,928 (38.0%) were White, and 3,710 (35.9 %) were others including Mexican American, other Hispanics or mixed races (**Table 1**). The result of this study showed that a total of 1,140 (11%) of 8954 participants had MetS showing 3 or more of risk factors defining MetS. The remaining 3785 (36.6%) of participants had 1 or 2 components of MetS and 4029 (38.9%) had no MetS.

# Table 1: Socio-demographic characteristics of the US population; data from NHANES 2007-2008

Variables	Characteristics	No (%)
Gender	Male	5080 (49.1)
	Female	5268(50.9)
Age in years	<20	5369(51.9)
	20-39.9	1923(18.6)
	40-59.9	1486(14.4)
	≥60	1570(15.2)
Race	African-American	2710(26.2)
	American-Whites	3928(38.0)
	Others	3710(35.9)

NB; others means Mexican American, other Hispanics or mixed races.

## Health and Morbidity characteristics

The NHANES 2007-2008 data of US population pointed, 3.7% of women were pregnant and forty percent of the population were Overweight/obese (BMI >= 25kg/m<sup>2</sup>). Among the study participants Vit-D deficiency and T2diabetes Mellitus were 33.1% & 5.0% respectively (**Table 2**).

Variables	Frequency	Percent	
Pregnancy status			
Yes	382	3.7	
No	2791	27.0	
Uncertain	200	1.9	
BMI			
Underwt (<18.5Kg/m²)	1892	18.3	
Normal (18.5 -24.9kg/m²)	2838	27.4	
Overwt/obese(>= 25kg/m <sup>2</sup> )	4150	40.1	
Vit-D deficiency			
No VitDD (VitD≥ 20 ng/ml)	4880	47.2	
VitDD( VitD < 20ng/ml)	3426	33.1	
T2Diabetes status			
No T2DM	9292	89.8	
T2DM	521	5.0	

Table 2: Hea	alth and m	norbidity cl	naracteristics	of US	Population	based on	NHANES
2007-2008.							

## Prevalence of Kidney Disease, Vitamin C deficiency and metabolic syndrome

Based on the survey 145(1.9%) and 1132(14.5%) of the US population had risk of kidney damage and developed metabolic syndrome (MetS≥3) respectively. In addition 366(5.1%) of the population were Vitamin C deficient as shown below (**fig.1**).



Figure 1: Prevalence of kidney disease, metabolic syndrome and relation with Vit-C deficiency in US population from NHANES 2007-2008.

## The association between vitamin C deficiency and metabolic syndrome

Collinearity diagnosis was tested using variance inflation factor (VIF) and the tolerance value, which was found to be acceptable to fit for continuous variables[23]. From the output the following equation was established as: ACR= $6.037-1.92^*$  vitamin C+ 0.76. This implies that for a unit decrease in serum vitamin C, the risk of developing kidney disease can be increased nearly by two folds (COR=-1.92; 95% CI:-3.41,-0.43) though it explained only 0.1 percent compared with the other un-explained variables (R<sup>2</sup>=0.001 and p value<0.01).

The binary logistic regression analysis was also undertaken to determine the strength of association between the outcome variable AU and the predictor variable MetS and the effect modifier VCD (**Table 2**). Keeping other variables constant, the risk of elevated AU was significantly associated with MetS more than with two folds (COR= 2.55; 95% CI: 1.76, 3.66). After adjusting for VCD alone, the odds ratio slightly increased (AOR=2.63; 95% CI (1.80, 3.85). After running regression with the interaction term the adjusted odds ratio was reduced (AOR=2.43; 95% CI: 1.61, 3.66). The severity of kidney disease measured by ACR was increased among individuals who developed both VCD and MetS (AOR=5.53; 95% CI: 2.73, 11.21); which indicating that vitC was an effect modifier between MetS and ACR.

Moreover, the following equations were developed from regression analyses after data weighting:

- 1. AU=-4.12+ 0.94  $\times$  MetS (crude)
- 2. AU=-4.31+ 0.97× MetS + 0.69 × VCD
- 3. AU=-4.28 + 0.89 × MetS (with VCD)
- 4. AU=-4.23 + 0.87 MetS (without VCD)
- 5. AU=-4.08+1.71×MetS\*VCD

Equation 1 shows that MetS was a risk for AU regardless of potential effect modifiers or confounders (p<0.001). Results from Equation 2 (see Table 2) show significant effects of MetS and VDD on AU (p<0.01). Equations 3 and 4 calculate the effect of MetS on AU accounting for the interaction with VCD. Computing the odds ratios from these coefficients ( $e^{0.89}$  (OR=2.43) for MetS effect on AU in the presence of VCD vs.  $e^{0.87}$  (OR=2.38) in the absence of VCD), the effect of MetS on AU was high among individuals who developed VitC deficiency compared with their counter parts.

Regardless of other covariates, VCD showed an increased risk of AU. In reference to 20-29.9, participants, ages greater than or equal to 60 years had increased odds of developing AU (p<0.001). The odds of AU increased with increasing age range. Higher age ranges had positive predictive power on AU. Whereas the predictive power of race was generally high among African-American.

Variables	Model 1: Bi-vari	ab Model 2: VCD	MetS*VCD	Model 3: race,
	analysis			gender & age
MetS	[1.76,3.66]	[1.80,3.85]	[1.61,3.66]	[1.24,1.87]
(>=3)	2.55***	2.63***	2.43**	1.89**
VCD	[1.35,4.20]	[1. 14,3.59]	[0.48,2.90]	[1.20,3.89]
(<0.25 mg/dl)	2.38**	2.03*	1.18 <sup>×</sup>	<b>2.16</b> <sup>*</sup>
Race	[0.99,2.17]			[1.17,2.81]
(African -American)	<b>1.46</b> ^			1.81**
Gender	[0.82,1.58]			[0.87,1.79]
(Female)	1.14 <sup>×</sup>			1.25 <sup>×</sup>
Age	[2.67,7.69]			[2.20,7.06]
(>=60 years)	4.53***			3.94***
MetS*VCD	[2.73,11.21]			
	5.53**			
BMI	[0.92,2.00]			[0.51,1.86]
	1.36 <sup>×</sup>			_
				1.12 <sup>×</sup>

Table 3: Logistic regression analysis indicated the strength of association betweenthe predictor variables and ACR.

Odds ratios reported; standard errors in parentheses

×p>0.20, ^p< 0.20, \*p< 0.05, \*\*p< 0.01, \*\*\*p< 0.001

## Discussion

In this study, we indicated that there is a positive association between MetS and AU worsened by VCD. We hypothesized that MetS predict AU and this association is significantly aggravated by VCD. It was evident from this study that the MetS and VCD interact in worsening the risk of AU as a measure of kidney disease. The association of MetS with AU remained significant after adjusting for VCD as an effect modifier and other potential confounders including body mass index (BMI) as a measure of adiposity, though it had insignificant relationship with ACR. Moreover, in the final regression analysis, we also considered the potential confounders such as: age, gender, and race. African Americans were at higher risk of developing metabolic abnormalities and the risk of kidney disease measured by ACR as compared with the white race. Moreover, old age individuals had also an increased risk of developing kidney disease as compared with the early adulthoods.

Eventhogh the relationship between MetS and kidney disease are biologically plausible[24], the pathophysiology how MetS causes kidney disease was not fully understood. However visceral adiposity with adipose tissue expansion as component of measuring MetS is highly interrelated with insulin resistance[25]. Insulin resistance is becoming the non-traditional risk factor in the casual pathway of kidney disease[26]. Insulin resistance is also associated with endothelial dysfunction, reduced synthase of endothelial nitric oxide, and worsening of renal hemodynamic function in conjunction with injury of podocytes resulting in hypertension and albuminuria[27]. Moreover, insulin resistance is associated with sodium retention, overproduction of low-density lipoprotein cholesterol, and hypertriglyceridemia, which may impair mitochondrial function and promote kidney cell damage[28].

However, it is the interest of the authors to warrant further investigation about the role of metabolic syndrome on the incidence and progression of kidney disease.

From this study, we evidenced that vitamin C deficiency (<0.25 mg/dl) worsens kidney disease among individuals who already developed metabolic syndrome. This hydrophilic antioxidant and specific cofactor for enzymes[29] had more effect in waist circumference and triglyceride which in turn improves the life of patients with MetS[30]. Vitamin C is also associated with an increased risk of endothelial dysfunction via an increase in oxidative stress among non-diabetic chronic kidney disease patients[31]. Animal studies also reported that vitC supplementation may maintain kidney morphology, renal function and decrease albuminuria[32] through the blockage of oxidative stress[33]. However, the exact biological mechanism how vitC worsens kidney disease among patients with MetS warrants further exploration.

Identification of the covariates while establishing the relationship between MetS and Kidney disease was also the interest of this study. Astonishingly, BMI was not significantly associated with ACR. However, linear measurement of body parts, and other crude measurement like that of BMI may not indicate the cause and effect relationship between the body fat accretion and the different cardio-metabolic syndromes[34]. Hence utilization of the standardized tools which may report the true body fat distribution had the paramount importance than the crude measurement of adiposity through BMI[35].

#### Strength and limitation of the study

This study was limited by cross sectional study in which cause-effect relationship couldn't be established[<u>36</u>, <u>37</u>]. So that longitudinal studies are needed to warrant further investigations to identify the combined impact of VCD and MetS on kidney disease. But the involvement of these clusters of disorders provides the important association between estimated dietary intake, obesity, body fat distribution, insulin action, inflammation, and endothelial, are all important in the development of kidney disease. Strength of the study is the large sample size from a validated data set.

#### Theoretical and practical implication

As described in many of the scientific literatures, investment in the preventive aspect of cardio - metabolic syndrome is one of the smartest activities to tackle further uremic syndromes. However, to invest on this, it demands a comprehensive package of monitoring and evaluation programs that will consider both community involved health education targeting life style modification which enhances increased consumption of vitC containing food groups or Vitamin C fortified diets.

Hence, specific considerations tailored to metabolic syndrome, vitC and kidney disease measured by ACR screening and monitoring mechanisms at the community level is critical to tackle further medical complications.

Despite there was a vast investments of resources to improve and decrease the burden of noncommunicable disease (NCDs) such as kidney disease, there was a meager evidence on how metabolic syndrome and vitamin C deficiency synergistically cause kidney disease.

#### Conclusion

The study demonstrates that vitamin C deficiency aggravates the risk of developing kidney disease among patients with MetS. Being old age and African-American individuals were more prone for kidney disease. So that, the role of vitC fortified foods on prevention of kidney diseases among patients with MetS warrant further investigation.

## Abbreviations

BMI, body mass index; CRP, C-reactive protein; DM, diabetes mellitus; MetS, metabolic syndrome; vitC, vitamin C; IR, insulin resistance; AU, albuminurea; VCD, vitC deficiency; NHANES, National Health and Nutrition Examination Survey. BP, blood pressure; CKD, chronic kidney disease; CVD, cardiovascular disease; WC, waist circumference; FG/FBG, fasting blood glucose; TG, triacylglycerol; HDL, high density lipoprotein; ACR, albumin creatinine ratio.

## Declaration

## Ethics approval and consent to participate

The data used in this study are de-identified public data available for secondary analysis. The study was exempted by the Internal Review Board of Wayne State University as the project does not constitute human participant research according to the definition codified in the Common Rule at 45 CFR and FDA regulations. The detailed survey procedures including Ethical approval, human subject consent, home or mobile center examinations, interviews and blood collections, are already available elsewhere[<u>38</u>].

#### **Consent for publication**

Not applicable

## Availability of data and material

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

## **Competing interests**

The authors have declared that there is no competing interest.

## Author Contributions

AN, BM,AK,TK,DA,YN,ST and AF developed the protocol and involved in the design, selection of study, data extraction, statistical analysis and developing the initial drafts of the manuscript. All authors involved in the preparation and revision subsequent manuscript's draft. AN,YN and BM prepared the final draft of the manuscript. All authors read and approved the final draft of the manuscript

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