Vitamin D Deficiency and Electrocardiographic Subclinical Myocardial Injury: Results from National Health and Nutrition Examination Survey-III

Short Title: Vitamin D deficiency and Subclinical Myocardial Injury

Muhammad Imtiaz Ahmad MBBS[1], Parag Anilkumar Chevli MBBS[1], Yabing Li MD[2], Elsayed Z. Soliman MD, MSc, MS[2,3]

1) Department of Internal Medicine, Section on Hospital Medicine, Wake Forest School of Medicine, Winston Salem, NC
2) Epidemiological Cardiology Research Center (EPICARE), Department of Epidemiology and Prevention, Wake Forest School of Medicine, Winston Salem, NC
3) Department of Internal Medicine, Section on Cardiology, Wake Forest School of Medicine, Winston Salem, NC

***Corresponding Author*:**

Muhammad Imtiaz Ahmad MBBS
Wake Forest School of Medicine
Medical Center Blvd, Winston Salem, NC 27157
Tel: 336-716-3674 Fax: 336-716-0030
E-mail: muahmad@wakehealth.edu

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Abstract

Objective: Association of cardiovascular disease (CVD) with non-traditional risk factors such as vitamin D deficiency has been examined previously. An investigation of the association of vitamin D with

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subclinical myocardial injury (SC-MI) based on an electrocardiographic score is a simple, cost-effective and innovative way to explore this relationship.

Methods: This analysis included 6079 participants (58.3±13.1 years; 54.1% women) without CVD from NHANES III. A multivariable logistic regression model was used to examine the association between vitamin D categories (<20 ng/ml, 20-29 ng/ml and >30 ng/ml(reference) and cardiac injury score (CIIS).

Results: There was an incremental increase in the prevalence of SC-MI across vitamin D categories with the highest prevalence in <20 ng/ml, followed by 20-29 ng/ml and then >30 ng/ml (trend p-value <.0001). There was a statistically significant association between vitamin D deficiency (<20 ng/ml) and SC-MI (OR(95%CI): 1.27(1.04-1.55), p = 0.04). This association was stronger in men than women (OR(95%CI): 1.74(1.32-2.30) vs. 0.94(0.70-1.25) respectively; interaction p-value 0.002).

Conclusions: Vitamin D deficiency is associated with SC-MI, especially in men. These findings may further highlight the role of non-traditional risk factors in the development of CVD. The value of vitamin D supplementation in the prevention of myocardial ischemia and injury may warrant investigation.

Key Words: Vitamin D deficiency, Subclinical Myocardial Injury, NHANES III

Introduction

Association between vitamin D deficiency and cardiovascular disease has been established through observational studies.[1,2] Vitamin D levels are usually measured by serum 25-hydroxyvitamin D (25[OH]D). Low levels of serum 25(OH)D are associated with increased prevalence of coronary heart disease[3,4] as well as incident cardiovascular disease.[5] Although, most studies have failed to show any benefit of vitamin D supplementation on cardiovascular outcomes, including myocardial infarction and stroke or cardiovascular risk factors (high lipids, glucose, blood pressure).[6-9] Thus, it is unknown whether there is any causal association between vitamin D and cardiovascular disease (CVD) and whether the association differs by age, sex, and race.

The Cardiac Infarction/Injury Score (CIIS) was developed as an electrocardiogram-based score to identify patients with previous myocardial infarction.[10] This score uses electrocardiographic features that often are missed with conventional criteria for the diagnosis of myocardial infarction or injury, such as abnormal T-wave amplitude and direction. Subclinical myocardial injury as defined by CIIS≥10, in those without manifestations of CVD has been associated with an increased risk of CVD and all-cause mortalities.[11] These findings highlight the important role of CIIS to identify subclinical myocardial injury (SC-MI).

To our knowledge, however, there have been no population-based studies evaluating the association of vitamin D deficiency and SC-MI using CIIS. Therefore, we sought to examine cross-sectional association between levels of serum 25(OH)D and SC-MI in a sample from the third National Health and Nutrition Examination Survey (NHANES III) free of CVD. We hypothesize that low 25(OH)D levels (<20 ng/mL) would be associated with prevalent elevated CIIS (≥10) independent of traditional CVD risk factors, lifestyle factors, and socioeconomic status.

Methods

Study Participants

We analyzed data from the Third National Health and Nutrition Examination Survey (NHANES III), a cross-sectional study conducted between 1988 and 1994 that used a multistage stratified clustered probability design to select a representative sample of the civilian non-institutionalized US population.[12] The NHANES III study was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB), and documented consent was obtained from participants. Between 1988 and 1994, initial home interviews were conducted to collect baseline information, including demographics (age, sex, race/ethnicity), medication data (e.g., use of antihypertensive and lipid-lowering medications), past medical history (e.g., history of CVD), and behavioral data (e.g., smoking). Subsequently, participants visited mobile examination centers and gave blood samples to record basic laboratory values for each participant (e.g., total cholesterol, plasma glucose).

Measurement of Vitamin D

In the NHANES-III, serum 25-OH vitamin D was measured by a radioimmunoassay(RIA) kit after extraction with acetonitrile (DiaSorin, Stillwater, MN) by the National Center for Environmental Health, Centers for Disease Control and Prevention (Atlanta, GA). The kit manufacturer reformulated the kit in the late 1990s by introducing an antibody that improved binding. To assess the magnitude of change of the reformulated assay on the originally measured 25-OH vitamin D in NHANES-III, the CDC laboratory reanalyzed a subset of 150 samples representative of the entire NHANES-III population using the reformulated assay, and the results were regressed using the following equation: 25-OH vitamin D (corrected 2004 RIA) = 0.8429 * 25-OH vitamin D (1988 –1994 RIA) + 2.5762 (nmol/L).[13] We categorized Vitamin D levels as 1) <20 ng/mL 2) 20-29 ng/mL and 3) ≥30ng/mL (reference).[14,15]

Measurement of Cardiac Infarction/Injury Score

Resting 12-lead electrocardiograms were obtained with a Marquette MAC 12 system (Marquette Medical Systems, Milwaukee, Wisconsin) during the mobile examination visits by trained technicians. Analysis of electrocardiograms was achieved through a computerized automated process and visual inspection by a trained technician located in a centralized core laboratory. The calculation CIIS and methodology have been described previously[10]. Briefly, CIIS is based on a weighted scoring system taking several objective electrocardiographic waveform components related to myocardial injury and ischemia, both discrete and continuous, and generating a risk-stratified scoring system. The score is defined by a combination of 11 discrete and 4 continuous features and provides a simple scoring scheme suitable for both visual and computer classification of a standard 12-lead electrocardiogram (ECG). By design, CIIS values were multiplied by a factor of 10 in NHANES III to avoid using decimal points. We reported CIIS values by dividing by 10. The SC-MI was defined as CIIS values $\geq$ 10 points.[10,11]

Measurement of Other Variables

In the home interview, demographic information regarding age (continuous in years), sex (male and female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American and other), income (<$20,000/year, >$20,000/year), smoking status (never, current, and former), leisure time physical activity (number of times engaged in physical activity in past month), were collected. Height was measured using a wall-mounted stadiometer, and weight was measured using a Toledo digital scale in minimal clothing. BMI was calculated from height and weight measurements. WC was measured at the iliac crest after a normal exhalation of breath. Insulin resistance was defined as fasting blood glucose $\geq$ 100 mg/dl, or history of diabetes or taking anti-diabetic medications. Blood pressure (mmHg) was measured three times during the in-home interview and three additional times during the participant's visit to the mobile examination center. Blood samples were collected via venipuncture by a phlebotomist. Samples were analyzed for total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C),

triglycerides (TG), serum creatinine, c-reactive protein (CRP), calcium, phosphorus, and glucose, using laboratory procedures as reported by NCHS.

For the purpose of this analysis, we only considered NHANES-III participants who underwent an ECG recording (n = 8561). We excluded participants with a history of CVD (myocardial infarction, heart failure, or stroke), or with electrocardiographic evidence of myocardial infarction or any major abnormalities on their electrocardiograms according to the Minnesota Code classification[16] or missing key covariates. After all exclusions (n = 2482), 6079 participants were included in the analysis.

Statistical Analysis

Baseline characteristics were compared across three vitamin D categories (≥30 ng/ml, 20-29 ng/ml, and <20ng/ml). Continuous variables were reported as mean ± standard deviation (SD) while categorical variables were reported as frequency and percentage. Analysis of variance (ANOVA) was used to compare the continuous variables while Chi^2 was used to compare the categorical variables. Multivariable logistic regression analysis was used to compute odds ratios and 95% confidence interval (CI) for the cross-sectional association between each vitamin D category (20-29, <20, and ≥30 (reference) and SC-MI. Using CIIS as a continuous variable, we performed multivariable linear regression analysis with Vitamin D categories (20-29, <20 and ≥30 (reference) as the independent variable and CIIS as the outcome variable to compute beta-coefficient an and 95% confidence interval as well as adjusted mean and standard error of CIIS. We calculated *p for trend* across vitamin D categories in the fully-adjusted model using multivariable linear regression model. In both approaches, Model 1 was adjusted for age, sex, race and socioeconomic status. Model 2 adjusted for model 1 plus smoking and physical activity, BMI, insulin resistance, hypertension, total and high-density cholesterol, C-reactive protein, serum creatinine and Model 3 adjusted for model 2 plus serum calcium and serum phosphorus.

As an additional analysis, we conducted subgroup analysis stratified by age (using 65 years as a cut point), sex and race (whites vs. non-whites). The models were adjusted in a similar fashion to model 3 as mentioned above.

All statistical analyses were performed using with SAS version 9.4 (SAS Institute Inc, Cary, NC) and p-values were considered significant if less than 0.05.

Results

This analysis included 6079 participants (mean age 58.3±13.1; 54.1% women, 50.3% non-Hispanic whites). **Table 1** shows baseline characteristics of participants by vitamin D categories. Participants with Vitamin D deficiency were more likely to be young, woman, non-white, current smoker, belonged to low income level and to have more CVD risk factors like high systolic blood pressure, higher body mass index, lower physical activity levels, higher C-reactive protein levels and higher prevalence of insulin resistance. SC-MI was present in 21.2% (n= 1293) participants. The prevalence of SC-MI was 19.5%, 21.1% and 23% in ≥ 30, 20-29 and <20 vitamin D category respectively **(Figure 1).**

Table 2 shows the results of multivariable logistic regression analysis, in the model adjusted for sociodemographic, there was a statistically significant association between 25(OH) D level 20-29 and SC-MI (OR (95% CI):1.18 (1.01-1.38), p =0.03). However, this association became non-significant in the model adjusted for CVD risk factors (OR (95% CI): 1.17 (0.98-1.39), p= 0.08) and in the model adjusted for all potential confounders (OR (95% CI): 1.15 (0.96-1.37), p = 0.11). 25(OH) D level <20 was significantly associated with SC-MI in model adjusted for sociodemographic (OR (95% CI) 1.50 (1.27-1.77), p <.0001) and this association remained statistically significant after adjustment for cardiovascular risk factor and mineral metabolism-related biomarkers (OR (95% CI) 1.27 (1.04-1.55), p = 0.04). There was a dose-response relationship between levels of 25(OH)D, and SC-MI manifested as lower odds in the study participant with 25(OH)D levels between 20-29 ng/ml compared to those with 25(OH)D levels <20 ng/ml **(Table 2).**

Using CIIS as a continuous outcome variable, multivariable linear regression analysis showed a similarly strong association of vitamin D deficiency with SC-MI as observed with the logistic regression model. 25(OH)D of <20 ng/ml was associated with higher injury score (β (95% CI): 5.9 (0.84-10.9), p =

0.02) in the fully adjusted model. 25(OH)D 20-29 ng/ml was also associated with higher CIIS, but results were not statistically significant **(Table 3).**

There was an incremental increase in mean CIIS across 25(OH)D categories with the higher score in <20 ng/ml category followed by 20-29 ng/ml category and then ≥30 ng/ml category (*trend p-value* 0.02) **(Table S1).**

In subgroup analysis, there was a strong association of vitamin D deficiency and SC-MI among men compared with women (OR (95% CI): 1.74(1.32-2.30) vs. 0.94(0.70-1.25) respectively; interaction p-value=0.002). The association was also stronger in older participants vs. young participants (OR (95% CI): 1.81(1.29-2.54) vs. 1.06(0.83-1.36) respectively but interaction p-value did not reach significance level. There was no significant interaction by race either. **(Table 4).**

Discussion

In this analysis from the NHANES III, we found that low serum vitamin D levels were significantly associated with electrocardiographic subclinical myocardial injury in a dose-response fashion. The association persisted despite adjustment for cardiovascular risk factor and mineral metabolism-related biomarkers. We found evidence of heterogeneities in the association between vitamin D levels and SC-MI among subgroups. There was statistically significant interaction by sex with men having a strong association of SC-MI with vitamin D deficiency compared with women. Also, the association between vitamin D level and SC-MI was stronger in older participants compared to young participants.

Few studies have examined the association between vitamin D and ECG abnormalities.[17-19] Tuliani et al. examined the association between vitamin D levels and major ECG abnormalities and found that vitamin D deficiency (25(OH)D < 20ng/mL) was associated with an increased prevalence of major ECG abnormalities.[17] Zhang et al. found no association between 25(OH)D and QT interval in NHANES

III.[18] This is the first study looking at the association between vitamin D level and CIIS as a surrogate marker for SC-MI.

We have observed a strong association of SC-MI with 25(OH)D deficiency among men in our study. Whether the association of 25(OH)D deficiency with CVD differs by sex is unclear. Some studies have reported the stronger association of 25(OH)D deficiency with CVD in women while others have observed a stronger association in men. Kendrick et al. performed a cross-sectional analysis of data from NHANES-III and examined the association between serum 25(OH)D levels and prevalence of CVD and found that male gender was an independent predictor of prevalent CVD in vitamin D deficient participants.[4] However, Verdoia et al. showed in a large cohort of patients undergoing coronary angiography that the decreased levels of 25(OH)D observed in women had a stronger association with the prevalence and extent of coronary artery disease (CAD).[20] Further studies are required to assess the impact of gender differences on the relationship between 25(OH)D levels and CVD.

The association between vitamin D and SC-MI may involve a broad spectrum of underlying mechanisms. The protective effect of vitamin D has been linked to the downregulation of the renin-angiotensin system, inflammatory cytokines, cardiac myocytes proliferation, and parathyroid hormone levels[5,21-24]. Even though in our study the association between vitamin D deficiency and CIIS attenuated after adjusting for cardiovascular risk factors, the fact that it remained significant suggests a possible direct link between vitamin D deficiency and SC-MI.

The application of CIIS in this study was to find individuals without clinical CVD but who were at an increased risk of CVD and all-cause mortalities. CIIS has been shown to predict CVD and all-cause mortalities among those without apparent CVD.[11] Other electrocardiographic markers which are also associated with increased mortality include QRS duration and minor Q waves, but they only rely on 1 or 2 data points.[25,26] In contrast, the application of a scoring system, such as CIIS may be a more sensitive indicator of myocardial injury. It has also been shown that the CIIS improves the accuracy of the standard 12-lead electrocardiogram to identify patients with SC-MI.[27]

The findings of our study bear particular relevance as they were derived from a cardiovascular disease-free cohort of the nationally representative sample. With vitamin D level as a surrogate marker for poor health, we may be able to identify patients who are at high risk for CVD with the use of a12-lead electrocardiogram and scores similar to CIIS, which due to its low cost and widespread availability, is an effective screening tool.

Certain limitations need to be taken into consideration in the interpretation of our study. First, we had only a single measurement of 25(OH)D that may not reflect long-term vitamin D status. Second, there is seasonal variation in vitamin D levels with peaks in August and troughs in February[28]. NHANES-III data lacks information about the time of the year when blood samples were drawn for measurement of vitamin D. Therefore, we are unable to account for the seasonal changes in vitamin D levels in NHANES-III, another limitation of the study. Third, our study design was cross-sectional, and therefore, a causal relationship between vitamin D and SMI could not be established. Some of the measurements like smoking and physical activity are self-reported and thus subjected to recall bias. Finally, we adjusted for several confounders, but residual confounding remains a possibility. Our study has many strengths. Our study included a community based and multiracial population and large sample size with better generalizability of the US population. Also, we were able to adjust for many potential confounders and mediators including lifestyle variables, CVD risk factors, and markers of mineral metabolism.

Conclusions

We have observed an association between vitamin D deficiency and SMI in this racially diverse population, especially in men. This may suggest a high risk of CVD with vitamin D deficiency. Future research should consider sex differences when exploring the relationship between vitamin D and CVD as well as when considering vitamin D supplementation.

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Figure 1.
Prevalence of SC-MI across Vitamin D Categories.

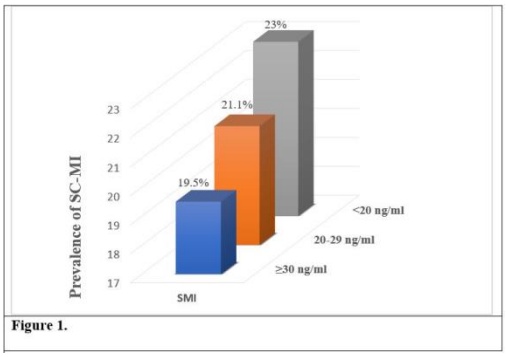

Figure 1.

Abbreviations: SC-MI, subclinical myocardial injury

Table 1. Baseline Characteristics of Study Participants

Characteristics **Mean± SD or n (%)**	**Vitamin D ≥30 ng/ml** **N=** 1935	**Vitamin D 20-29 ng/ml** N= 2185	**Vitamin D <20 ng/ml** N= 1959	***P*-Value†**
Age (years)	59.5±13.0	58.7±13.1	56.7±12.9	<.0001
Male (%)	1076(55.6%)	1012(46.3%)	700(35.7%)	<.0001
Race				<.0001
Non-Hispanic White	1343(69.4%)	1141(52.2%)	575(29.3%)	
Non-Hispanic Black	171(8.8%)	408(18.6%)	752(38.3%)	
Mexican American	352(18.1%)	537(24.5%)	545(27.8%)	
Other	69(3.5%)	99(4.5%)	87(4.4%)	
Total Annual Family Income <20,000	757(39.6%)	976(45.5%)	929(48.4%)	<.0001
Systolic Blood Pressure (mmHg)	130.2±18.6	131.4±18.7	131.7±19.5	0.03
Diastolic Blood Pressure (mmHg)	76.1±9.6	76.2±10.0	76.6±10.2	0.19
Insulin resistance (%)	681(35.2%)	885(40.5%)	827(42.3%)	<.0001
Total cholesterol (mg/dl)	218.9±43.3	217.9±43.1	215.8±45.7	0.07
HDL Cholesterol	51.3±16.2	51.1±15.6	52.2±7.6	0.06
Serum Creatinine (mg/dl)	1.1±0.3	1.1±0.4	1.1±0.4	<.0001
Serum Total Calcium (mg/dl)	9.3±0.4	9.3±0.4	9.2±0.5	0.008
Serum Phosphorous (mg/dl)	3.4±0.5	3.4±0.5	3.4±0.5	0.0001
Vitamin D Supplements (%)	638(32.9%)	564(25.8%)	301(15.3%)	<.0001
C-reactive protein (mg/dl)	0.45±0.8	0.46±0.6	0.54±0.7	<.0001
Body mass index (Kg/m^2)	26.6±4.6	27.6±5.2	28.6±6.3	<.0001
Smoking (%)				
Current Smoker	371(19.1%)	464(21.2%)	559(28.5%)	<.0001
Former Smoker	741(38.2%)	663(30.3%)	528(26.9%)	0.001
Never Smoker	823(42.5%)	1058(48.4%)	872(44.5%)	0.0005
Physical Activity (METs per week) *	24.4(8.3-40.6)	17.3(5.8-34.8)	12.0(4.5-29.0)	<.0001
Subclinical Myocardial Injury (%)	379(19.5%)	463(21.1%)	451(23.0%)	0.03
Cardiac Injury Score	5.05±6.61	5.14±6.70	5.42±6.96	0.21

†p-value as calculated by ANOVA for continuous and chi^2 for categorical variables.
***METs reported as median and IQR.**
Insulin resistance defined as fasting blood glucose ≥100 mg/dl, or history of diabetes or taking medications
Abbreviations: HDL, high-density cholesterol; MET, metabolic equivalent

Table 2. Multivariable Odds Ratio and 95% CI of association between Vitamin D Categories and Subclinical Myocardial Injury

Vitamin D Categories	Model 1		Model 2		Model 3	
	Odds Ratio (95% CI)	***p-value***	***Odds Ratio (95% CI)***	***p-value***	***Odds Ratio (95% CI)***	***p-value***
Vitamin D ≥30 ng/ml	*Reference*		*reference*		*reference*	
Vitamin D 20-29 ng/ml	1.18(1.01-1.38)	0.03	1.17(0.98-1.39)	0.08	1.15(0.96-1.38)	0.11
Vitamin D <20 ng/ml	1.50(1.27-1.77)	<.0001	1.30(1.07-1.59)	0.02	1.27(1.04-1.55)	0.04

Model 1 adjusted for age, sex, race and socioeconomic status
Model 2 adjusted for model 1 plus smoking and physical activity, BMI, insulin resistance, hypertension. total and high-density cholesterol, C-reactive protein, serum creatinine.
Model 3 adjusted for model 2 plus serum calcium and serum phosphorous
Hypertension defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 or taking antihypertensive medications
Subclinical myocardial injury defined as cardiac injury score ≥10.

Table 3. Multivariable Beta-coefficient and 95% CI of association between Vitamin D Categories and CIIS.

Vitamin D Categories	Model 1*		Model 2†		Model 3‡	
	Beta-Coefficient (95% CI)	***p-value***	***Beta-Coefficient (95% CI)***	***p-value***	***Beta-Coefficient (95% CI)***	***p-value***
Vitamin D ≥30 ng/ml	*Reference*		*reference*		*reference*	
Vitamin D 20-29 ng/ml	3.2(-0.9-7.3)	0.12	1.8(-2.72-6.3)	0.43	1.6(-2.8-6.2)	0.47
Vitamin D <20 ng/ml	10.1(5.76-14.4)	<.0001	6.1(1.13-11.2)	0.01	5.9(0.84-10.9)	0.02

***Model 1 adjusted for age, sex, race and socioeconomic status**
†Model 2 adjusted for model 1 plus smoking and physical activity, BMI, insulin resistance, hypertension, total and high-density cholesterol, C-reactive protein, serum creatinine.
‡Model 3 adjusted for model 2 plus serum calcium and serum phosphorous
Hypertension defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 or taking antihypertensive medications
Abbreviations: CIIS, cardiac injury score

Table 4. Multivariable Odds Ratios and 95% CI for the association between vitamin D categories and Subclinical Myocardial Injury in Subgroups

	Vitamin D Categories	Odds ratio (95% CI)	Interaction P-value
Male	Vitamin D 20-29 ng/ml	1.30(1.02-1.67)	0.002
	Vitamin D <20 ng/ml	1.74(1.32-2.30)	
Female	Vitamin D 20-29 ng/ml	0.97(0.75-1.27)	
	Vitamin D <20 ng/ml	0.94(0.70-1.25)	
Whites	Vitamin D 20-29 ng/ml	1.23(0.99-1.53)	0.42
	Vitamin D <20 ng/ml	1.21(0.91-1.62)	
Non-Whites	Vitamin D 20-29 ng/ml	1.01(0.73-1.39)	
	Vitamin D <20 ng/ml	1.22(0.89-1.66)	
Age >65 years	Vitamin D 20-29 ng/ml	1.36(1.02-1.82)	0.69
	Vitamin D <20 ng/ml	1.81(1.29-2.54)	
Age ≤65 years	Vitamin D 20-29 ng/ml	1.04(0.83-1.31)	
	Vitamin D <20 ng/ml	1.06(0.83-1.36)	

Reference group= Vitamin D≥30 ng/ml
Model adjusted for Age, Sex, Race, Socioeconomic status, Smoking and Physical Activity, BMI, insulin resistance, hypertension, total and high-density cholesterol, C-reactive protein, serum creatinine, serum calcium and serum phosphorous
Hypertension defined as systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 or taking antihypertensive medications