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Sleep-disordered breathing is associated with higher carboxymethyllysine level in elderly women but not elderly men in the cardiovascular health study

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Abstract

Context—Carboxymethyl-lysine (CML) results from oxidative stress and has been linked to cardiovascular disease.

Objective—To investigate the association between sleep-disordered breathing (SDB) - a source of oxidative stress- and CML.

Materials and Methods—1002 participants in the Cardiovascular Health Study (CHS) were studied.

Results—Women with SDB had significantly higher CML concentration compared with those without SDB (OR=1.63, 95%CI=1.03 – 2.58, p=0.04). The association was not significant among men.

Discussion—SDB was associated with CML concentration among elderly women but not men in the Cardiovascular Health Study.

Conclusion—Accumulation of CML may be an adverse health consequence of SDB

Keywords

Carboxymethyl-lysine; Advanced Glycation End-Product; Sleep-Disordered Breathing; Oxidative stress; Apnea-Hypopnea Index

INTRODUCTION

Advanced glycation end-products (AGEs) have been implicated in pathologies such as diabetic microvascular disease, rheumatoid arthritis, Alzheimer's disease, end-stage renal disease, hypertension, atherosclerosis and other late-life disorders[1]. Major AGEs include hydroimidazolone (HI), Nε-carboxymethyl-lysine (CML), pentosidine (PTD), and glucosepane[1]. CML is a general biomarker of oxidative stress and damage in tissue proteins. It is formed on protein by combined nonenzymatic glycation and oxidation reactions, and also during metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein[2]. CML has serious implications for cardiovascular disease. This dominant AGE in tissue proteins is detectable in atherosclerotic plaques, where it can lead to trapping of LDL particles and trigger pro-inflammatory cascades through binding to the receptor for AGEs (RAGE)[2–4]. Previous research from our group[5] and others[6] have indeed linked circulating CML levels with risk of cardiovascular events. However, the modifiable determinants of CML remain incompletely characterized.

Sleep-disordered breathing (SDB) is accompanied by production of free radicals and increased oxidative stress, and hence represents an attractive risk factor for CML accumulation. In sleep-disordered breathing episodes of hypoxia occur repeatedly, which impair production of adenosine triphosphate (ATP), up-regulate glycolysis, and consequently produce reactive oxygen species (ROS)[7, 8]. Because sleep-disordered

breathing, especially obstructive sleep apnea, can be treated by the use of continuous positive airway pressure (CPAP),[9–12] its role as a potentially modifiable risk factor is especially important.

Few studies have investigated the effect of sleep-disordered breathing on AGEs, [12, 13]. One pilot study found that continuous positive airway pressure treatment reduces serum AGEs in patients with obstructive sleep apnea syndrome[14]. Another clinical study showed that patients with SDB have increased lipid peroxidation biomarkers, an indication of increased state of oxidative stress, which improves with CPAP[15]. However, no large-scale studies of SDB and CML exist to our knowledge. As a result, the main aim of our study was to investigate the association between sleep-disordered breathing and serum CML levels in the Cardiovascular Health Study, a population-based study of older Americans. We hypothesized that SDB would be associated with higher concentrations of CML.

METHODS

Study Participants—The subjects for this population-based cross-sectional study were CHS cohort members who were enrolled in the SHHS. The CHS is a population-based longitudinal study designed to determine the risk factors for development and progression of cardiovascular disease (CVD) in older adults (≥65 years) in the United States. The CHS recruited 5,888 adults from four U.S communities: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania[16]. The SHHS is a prospective cohort study designed to investigate obstructive sleep apnea (OSA) and other sleep-disordered breathing (SDB) as risk factors for the development of cardiovascular disease. Participants in SHHS were recruited from existing cohort studies: Atherosclerosis Risk in Communities Study, CHS, Framingham Heart Study, Strong Heart Study, New York Hypertension Cohorts, and Tucson Epidemiologic Study of Airways Obstructive Diseases and the Health and Environment Study. Participants in the SHHS underwent home polysomnography (PSG) to assess the presence of OSA and other SDB[17].

Assessment of Sleep Disordered Breathing

All SHHS participants underwent a baseline home PSG between 1995 and 1997. SDB was measured by apnea-hypopnea index (AHI). AHI represents the number of respiratory events scored per hour of sleep. Apneas and hypopneas were classified according to the American Academy of Sleep Medicine criteria. An event was classified as apnea if there was a drop in peak thermal sensor excursion by ≥90% from baseline, for 10 seconds or greater, and an event was classified as hypopnea if there was a ≥30% drop in the nasal pressure excursion for 10 seconds or greater associated with ≥ 4% desaturation [18, 19]. We used the clinical cutpoint for AHI of 5 events per hour to classify SDB but tested the full dose-response in secondary analyses.

Measurement of CML

CML was measured as described by Kizer et al[5]. Briefly, fasting samples were stored at −80 degrees Celsius and CML measured using a competitive enzyme-linked immunosorbent

assay (AGE-CML ELISA, Microcoat, Penzberg, Germany). This immunoassay has similar affinity for protein-bound, peptide-bound, and free CML. Inter-assay coefficients of variation were 7–11%.

Selection and Definition of Covariates

The following variables were included based on expected associations with either SDB or circulating CML: age, sex, race, smoking, alcohol intake, body-mass index (BMI), hypertension, diabetes, estimated glomerular filtration rate (eGFR), C-reactive protein, hemoglobin, HDL cholesterol, prevalent myocardial infarction (MI), heart failure, coronary heart disease (CHD), stroke, and claudication. Age was classified as <75, 75–79, and 80 years or more. Race and ethnicity was classified as “White”, “African American or Black”, and “other”. The “other” class includes American Indian or Alaskan native, Asian or Pacific Islander. BMI was categorized according to the World Health Organization guidelines as “normal” (BMI <25.0 kg/m^2), “overweight” (BMI 25–29.9 kg/m^2), or “obese” (BMI ≥30 kg/m^2). Results did not change when BMI was treated as a continuous variable. Smoking status was categorized as “nonsmoker”, “former smoker”, or “current smoker”, and blood pressure was categorized into “normal”, “prehypertension”, or “hypertension” according to the Seventh report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC VII) criteria[20]. Diabetes status was defined based on American Diabetes Association/World Health Organization guidelines using glucose values measured after fasting and following a 2-hour oral glucose tolerance test.

Inclusion and Exclusion Criteria

A total of 1248 CHS subjects were enrolled in the SHHS for sleep testing between 1995 and 1997. These and additional CHS participants had CML values measured at the 1996–1997 visit (n=3373). This study includes 1002 subjects who successfully underwent polysomnography and had CML measured.

Statistical Analysis

Apnea-Hypopnea Index (AHI) was used on both continuous and dichotomized scales in this study. For the dichotomized scale, AHI was categorized as <5 and ≥5 events/hour. CML was skewed to the right and was log transformed before analysis; this generally appeared to improve model fit. The log transformed values were further winsorized at the first and 99^{th} percentiles to reduce the influence of extreme values. The cross-sectional relationship between SDB (assessed by AHI) and CML concentration was modeled in two ways: firstly with CML as a continuous variable using linear regression and secondly, to ensure robustness, CML was analyzed as a dichotomous variable using logistic regression where high CML represented the upper 20% of the CML distribution. The following variables were adjusted for: age, sex, race, smoking, alcohol intake, BMI, hypertension, estimated glomerular filtration rate (eGFR), C-reactive protein, hemoglobin, HDL cholesterol, prevalent MI, CHD, stroke, peripheral artery disease, and diabetes status. This parsimonious set of variables was arrived at through a model building technique recommended originally by Hosmer and Lemeshow[21], and described further by Jewell[22]. Stratified analyses based on gender were performed with adjustment for the confounders mentioned above. In addition, cubic splines and piecewise models were used to further evaluate the association between

AHI and CML. Measurements from previous years were used to impute missing values. All analyses were performed using SAS version 9.3. Levels of significance of all analyses were based on p-value of 0.05.

This study was approved by the Institutional Review Board (IRB) of the University of Louisville and executed after Data and Materials Distribution Agreement between the University of Louisville and the Cardiovascular Health Study.

RESULTS

The mean CML concentration among study participants was 6161.2 ng/ml, and participants experienced on average 11.5 apneas and hypopneas per hour of sleep (SD=12.6). The mean apneas and hypopneas per hour of sleep was 9.5 (SD=11.0) among women, and 14.1 (SD=14.1) among men.

Characteristics of participants according to SDB are shown in Table 1. BMI, alcohol use, MI, CHD, and diabetes were directly associated with SDB, while HDL was inversely associated with SDB. Compared to females, males were more likely to have sleep-disordered breathing.

The association between sleep disordered breathing and CML concentration was not significant in the entire study sample [Table 2]. However, we observed apparent effect modification by gender on the association between SDB and CML (p=0.06). Among women, we observed a 2% increase in CML for every unit increase in AHI (p=0.09) [Table 2]. Women with AHI ≥5 events/hr had a 6% significantly higher CML concentration compared with those with AHI <5 events/hr (p=0.01) [Table 2]. Percentage changes in CML were used instead of the values because CML was log-transformed. There was no significant association between SDB and CML among men [Table 2].

As a check for these findings, we dichotomized CML at the upper 20% of the CML distribution. Among women a 1-unit increase in AHI was associated with an odds ratio of 1.26 ng/ml (0.99 – 1.61) for an elevated CML (p=0.06) [Table 3], and women with AHI ≥5 events/hr were significantly more likely to have a high CML level compared with those with AHI <5 events/hr (OR=1.63, 95%CI=1.03 – 2.58, p=0.04) [Table 3]. There was no significant association between SDB and CML among men in the logistic regression analysis [Table 3].

To determine if the sex interaction might reflect interaction with other characteristics that differ by sex, we performed analyses that included interaction terms for both SDB with sex and SDB with cigarette smoking, physical activity, or hemoglobin level, all of which are higher among men. In all three cases, the interaction of SDB with sex remained largely unchanged. Similarly, we investigated whether BMI or age modified the effect of SDB on CML but no effect modification was found (P-values were 0.98 for age and 0.43 for BMI).

To determine whether a threshold in the association of SDB with CML exists, we further evaluated the association between AHI and CML using cubic splines and piecewise models.

CML was significantly associated with AHI up to a value of approximately 10 events/hr (p=0.03), but the association plateaued with AHI values above 10 events/hr (p=0.84).

DISCUSSION

In this study of over 1000 older adults with systematic PSG studies and measured CML concentrations, we did not detect a significant association between sleep disordered breathing (SDB) and carboxymethyl-lysine (CML) concentration in the entire study sample. However, the association between SDB and CML was modified by gender. Among women, SDB significantly increased CML level while the association was not significant among men.

This study is the first study investigating the association between SDB and CML. However, a few studies have investigated the association between SDB and advanced glycation end-products. In a study conducted by Lam et al.,[12] among 105 men (mean age 43.5 years) without diabetes, significantly higher serum advanced glycation end-product (AGE) levels were observed in subjects with SDB (AHI ≥5), compared to subjects without SDB (AHI <5). Among subjects with moderate to severe SDB who underwent CPAP treatment, serum levels of advanced glycation end-products decreased suggesting a direct relationship between SDB and advanced glycation end-products. Tan et al.,[13] investigated the association between SDB and advanced glycation end-products in a matched study involving 119 nondiabetic patients with SDB (AHI≥5) and 243 healthy controls. They found that markers of oxidative stress and advanced glycation end-products were higher in SDB patients. Although the study by Tan et al, involved both men and women their SDB cases consisted largely of men (81%).

The studies by Tan et al., and Lam et al. were consistent in identifying significant associations between SDB and advanced glycation end-products. We studied a much older group and found a significant association between SDB and CML among women but not among men. CML is a well-characterized advanced glycation end-product, and has been described as a general biomarker of oxidative stress. Hence, a direct relationship between SDB and CML was expected as found among women. Although the exact explanation for our sex discrepancy is uncertain, we suspect survival bias may play a role in the null effect of SDB on CML among the elderly men in this study. Life expectancy for men and women in the United States is approximately 76.4 years and 81.2 years, respectively[23]. We studied a population with mean age of 77.7 years for men and 77.4 years for women, arguing that these men were already selected for longevity. It seems plausible that men in this study could reflect an unusual group of survivors who might be resistant to harmful effects of SDB. Hence, the CML values of these men may not reflect what would be observed if younger population were studied where differences in survival between men and women would be more consistent.

We also evaluated whether sex differences in lifestyle factors, BMI, hemoglobin, and age might explain the sex interaction, and found none.

As we continue to explore the possible reasons for the sex differences, it is worth noting that our splines suggest dichotomization of AHI at approximately 10 among women. AHI values above this threshold did not appear to influence CML levels further among women. Although the AASM diagnostic criteria for SDB classify AHI ≥5 as abnormal, our results suggest that an AHI of 10 or higher poses maximal risk and may represent a vulnerable group well-suited for targeting interventions.

Strengths of this paper include firstly, the reliability of the data. CHS has extensive clinical data on participants coming from four U.S communities with repeated measures of covariates and frozen specimens. All participants underwent routine home PSG, avoiding bias related to clinical referrals. To our knowledge, this is, by far, the largest study of SDB and an AGE yet described. Limitations of this study include its cross-sectional nature, as we only measured CML at one CHS visit. Hence, we are unable to assess the directionality of any observed associations. Repeated CML measurements with time may help to retrospectively study longitudinal effects of SDB on CML accumulation. Although large, this study included only 1002 participants, and a larger sample may be instrumental in establishing the validity of the findings, particularly for subgroups of men and women. By design our study involved only elderly individuals. These results may not apply to a younger age group. The sex interaction found in this study needs to be replicated in other studies.

CONCLUSION

CML levels have been linked to diseases of aging, particularly cardiovascular disease. Findings from this study indicate that CML level depends on interplay between gender and sleep-disordered breathing, at least in older adults. Sleep-disordered breathing significantly influenced CML concentration among elderly women but not men. These findings suggest the need for further studies to test whether treatment of SDB reduces CML accumulation and its health consequences.

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IMPLICATIONS

Sleep without nocturnal desaturations is important for good health among people of all ages. However, findings of this study suggest greater consequences for elderly women. The advanced glycation end-product, CML is a primary contributor to atherosclerotic plaque. The high level of CML, among older women with sleep-disordered breathing as found in this study, has consequences for cardiovascular disease as well as other diseases of aging among this group. Aging in itself affects health and normal functioning and it only gets worse if care is not taken to reduce levels of metabolic wastes such as CML. Public health professionals, physicians, nurses, and senior caregivers have roles to play in encouraging screening, diagnosis and treatment of SDB in elderly in order to reduce CML accumulation and promote healthy aging, particularly among women.

Table 1

Characteristics of up to 1002 participants from the Cardiovascular Health Study by Apnea-Hypopnea Index

		Apnea-Hypopnea Index (Events/hour)		
Variable	**N**	**<5 (n=396)N=396**	**≥5 (n=606)**	**P-value**
Age		**N (%)**		
<75	263	114 (43.4)	149 (56.7)	
75–79	455	174 (38.2)	281 (61.8)	
≥80	284	108 (38.0)	176 (62.0)	0.34
Gender				
Male	429	130 (30.3)	299 (69.7)	
Female	573	266 (46.4)	307 (53.6)	<.0001
Race				
White	816	313 (38.4)	503 (61.6)	
Black/AA	179	82 (45.8)	97 (54.2)	
Other	7	1 (14.3)	6 (85.7)	0.07
BMI (kg/m^2)				
Normal	311	147 (47.3)	164 (52.7)	
Overweight	464	175 (37.7)	289 (62.3)	
Obese	215	70 (32.6)	145 (67.4)	0.002
Smoking Status				
Non-smoker	485	196 (40.4)	289 (59.6)	
Former smoker	449	169 (37.6)	280 (62.4)	
Current smoker	63	31 (49.2)	32 (50.8)	0.19
Blood Pressure Status				
Normal	351	140 (39.9)	211 (60.1)	
Prehypertension	126	50 (39.7)	76 (60.3)	
Hypertension	522	204 (39.1)	318 (60.9)	0.97
MI Status				
No	904	370 (40.9)	534 (59.1)	
Yes	98	26 (26.5)	72 (73.5)	0.006
CHD				
No	761	317 (41.7)	444 (58.3)	
Yes	241	79 (32.8)	162 (67.2)	0.01
Stroke				
No	962	380 (39.5)	582 (60.5)	
Yes	40	16 (40.0)	24 (60.0)	0.95
Claudication				
No	974	386 (39.6)	588 (60.4)	
Yes	28	10 (35.7)	18 (64.3)	0.68
Diabetes Status (ADA)				
Normal	754	316 (41.9)	438 (58.1)	
IFG	112	38 (33.9)	74 (66.1)	

		Apnea-Hypopnea Index (Events/hour)		
Variable	**N**	**<5 (n=396)N=396**	**≥5 (n=606)**	**P-value**
Diabetic	131	40 (30.5)	91 (69.5)	0.02
Alcohol (g) Mean (S.D)	988	3.4 (6.6)	5.0 (11.3)	0.01
HDL (mg/dl)	982	55.1 (15.2)	52.3 (13.7)	0.003
Hemoglobin (g/dl)	978	13.7(1.4)	13.8 (1.3)	0.37
C-reactive protein (mg/L)	972	5.2 (9.9)	4.8 (8.8)	0.55
eGFR-cysC	925	83.6(19.0)	81.6 (18.2)	0.11

Table 2

The effect of SDB (AHI) on CML by GENDER (**Linear Regression**: CML concentration as continuous variable)

Parameter	N	Estimate log(ng/ml)	SE	Pr >|t|
ALL				
AHI <5	396	Referent		
AHI ≥5	606	0.03	0.02	0.14
AHI (continuous)	*1002*	*0.004*	*0.009*	*0.65*
MALE	**429**			
AHI <5	130	Referent		
AHI ≥5	299	−0.02	0.03	0.42
AHI (continuous)	*429*	*−0.02*	*0.01*	*0.18*
FEMALE	**573**			
AHI <5	266	Referent		
AHI ≥5	307	**0.06**	**0.02**	**0.01**
AHI (continuous)	*573*	*0.02*	*0.01*	*0.09*

Models were adjusted for age, BMI, gender, smoking, alcohol, HDL, hemoglobin, C-reactive protein, eGFR-Cystatin C, CHD, MI, Claudication, Stroke, diabetes, hypertension, race.

P-values for interaction

AHI (continuous)*Gender = 0.06

AHI (dichotomized)*Gender = 0.06

Table 3

The effect of SDB (AHI) on CML by GENDER (**Logistic Regression**: "High" CML = upper 20% of the CML distribution)

SDB	N	Odds Ratio	95% CI	P-value
ALL				
AHI <5	396	Referent		
AHI ≥5	606	1.34	0.93 – 1.93	0.12
AHI (continuous)	*1002*	*1.08*	*0.90 – 1.30*	*0.41*
MALES	**429**			
AHI <5	130	Referent		
AHI ≥5	299	1.00	0.53 – 1.88	1.00
AHI (continuous)	*429*	*0.87*	*0.64 – 1.17*	*0.36*
FEMALES	**573**			
AHI <5	266	Referent		
AHI ≥5	307	1.63	1.03 – 2.58	0.04
AHI (continuous)	*573*	*1.26*	*0.99 – 1.61*	*0.06*

Models were adjusted for age, BMI, gender, smoking, alcohol, HDL, hemoglobin, C-reactive protein, eGFR-Cystatin C, CHD, MI, Claudication, Stroke, diabetes, hypertension, race.

P-values for interaction

AHI (continuous)*Gender = 0.06

AHI (dichotomized)*Gender = 0.27