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Serum Carboxymethyl-lysine, an Advanced Glycation End Product, and Age-Related Macular Degeneration: the Age Gene/ Environment Susceptibility-Reykjavik Study

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Abstract

Importance—Advanced glycation end products have been implicated in the pathogenesis of age-related macular degeneration (AMD).

Objective—To investigate the relationship between serum carboxymethyl-lysine (CML), a major circulating advanced glycation end product, and AMD in older adults.

Design—Cross-sectional study.

Setting—Population-based sample of older adults in the Age Gene/Environment Susceptibility-Reykjavik Study.

Participants—4907 adults, aged ≥66 years

Exposure—Serum CML and risk factors for AMD.

Main Outcome Measures—Early or late AMD, assessed through fundus images taken through dilated pupils using a 45-degree digital camera and grading for drusen size, type, area, increased retinal pigment, retinal pigment epithelial depigmentation, neovascular lesions, and geographic atrophy using the modified Wisconsin Age-Related Maculopathy Grading System.

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The authors have no conflicts of interest.

Results—Of the 4907 participants, 1025 (20.9%) had early AMD and 276 (5.6%) had late AMD. Mean (standard deviation [SD]) serum CML concentrations among adults with no AMD, early AMD, and late AMD (exudative AMD and pure geographic atrophy) were 3.0 (0.9), 3.1 (1.0), and 3.1 (0.9) μmol/L, respectively (P = 0.07). Log serum CML (per 1 log SD) was not associated with any AMD (early and late AMD) (Odds Ratio [O.R.] 0.97, 95% Confidence Interval [C.I.] 0.90, 1.04, *P* = 0.44) or with late AMD (O.R. = 0.94, 95% C.I. 0.82, 1.08, *P* = 0.36) in respective multivariable logistic regression models adjusting for age, sex, body mass index, smoking, and renal function.

Conclusion—Higher serum CML had no significant cross-sectional association with prevalent AMD in this large population-based cohort of older adults in Iceland.

Keywords

advanced glycation end products; age-related macular degeneration; aging; Iceland

Age-related macular degeneration (AMD) is the leading cause of visual loss among adults aged 65 or older in developed countries.[1] With the growing population of older adults, the prevalence of advanced AMD is projected to increase by 50% to nearly 3 million in 2020 in the U.S. alone.[2] The global cost of visual impairment due to AMD alone was an estimated $343 billion in 2010, including $255 billion in direct health care costs.[3] Lifestyle and dietary modifications, intravitreal antiangiogenic therapy, and antioxidant supplementation are among the current strategies to reduce the morbidity of AMD.[1] Despite advances in treatment and prevention, AMD has no effective cure and remains the primary cause of irreversible blindness in older adults.

The pathogenesis of AMD has been linked to mechanisms involving inflammation/innate immune dysregulation and oxidative stress. Age is a strong risk factor for AMD, with the prevalence of advanced AMD increasing from about 0.2% in ages 55-64 y to 13% in those >85 y.[4] Smoking,[5-7] obesity, white race,[8] and low intake of dietary antioxidants[9] and omega-3 fatty acids[10] are associated with an increased risk of AMD. There is a strong genetic susceptibility to AMD as shown in twin studies,[11] familial aggregation analyses,[12] and a large and growing body of association studies that have identified several common AMD-associated variants, for example, in and around complement factor H and the *ARMS2/HTRA1* region.[13-17] Other studies have implicated lipid metabolism genes such as apolipoprotein E, hepatic lipase, cholesterol ester transfer protein, lipoprotein lipase, and very low density lipoprotein receptor, and extracellular matrix genes such a hemicentin1 and fibulin 5 in AMD risk.[16,17] Although variants within identified major susceptibility genes to AMD play a role in over half of AMD cases, many individuals carrying AMD risk genotypes never develop the disease, and only a fraction with diagnosed disease progress to advanced AMD with visual loss.

Advanced glycation end products (AGEs) are a heterogeneous group of bioactive molecules formed by the non-enzymatic glycation of proteins, lipids, and nucleic acids. AGEs are implicated in a wide number of adverse aging-related outcomes, including cardiovascular disease, diabetes, chronic kidney disease, osteoporosis, and sarcopenia.[18] AGEs alter the structural integrity of tissues by cross-linking collagen and are thought to upregulate

inflammation through binding with the receptor for AGEs (RAGE).[18] AGEs are implicated in the pathogenesis of AMD through various lines of evidence. Immunohistochemical studies have shown accumulation of AGEs such as pentosidine in the Bruch membrane with increasing age,[19] carboxymethyl-lysine (CML) in drusen of eyes with AMD,[20] and of AGEs and RAGE in photoreceptors and retinal pigment epithelium (RPE) of eyes with AMD.[21] Basal laminar deposits, which develop between the RPE cells and the basement membrane and are specific for AMD, show greatly increased expression of RAGE.[22] A key factor in the pathogenesis of neovascular AMD is the expression of vascular endothelial growth factor (VEGF). The activation of RAGE leads to the increased expression of VEGF via the activation of NF-κB.[23] One study suggested that plasma CML and pentosidine concentrations were higher in 58 patients with AMD compared with 32 controls,[24] but further corroboration is needed.

We hypothesized that elevated circulating CML is independently associated with AMD in older adults. To address this hypothesis, we measured serum CML and assessed its relationship with AMD in a large, population-based cohort of older adults in Iceland.

Methods

Study subjects

The Age, Gene/Environment Susceptibility (AGES) Reykjavik Study is a population-based study aimed to investigate genetic and environmental factors contributing to health, disability and disease in older people, including systemic disease as well as eye disease. The study design and assessment of the cohort have been described elsewhere.[25] In 2002, when the AGES Reykjavik Study began, 11,549 previously examined cohort members of the Icelandic Heart Association's Reykjavik cohort (1967–1996) were still alive according to the Icelandic Census Database, and a random sample of 5,764 individuals were examined for the AGES – Reykjavik Study in 2002-2006.[25] The comprehensive AGES protocol required each participant to complete three visits to the Icelandic Heart Association (IHA) Research Center, within a window of 3–6 months. The ocular component was included as part of the third visit in which 5,330 persons participated. As a part of the assessments at the IHA Research Center a questionnaire was administered, visual acuity was assessed, and images were acquired from the retina.[26] Fundus images were available from 5272 individuals for the determination of AMD status. The AGES – Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00–063), which acts as the Institutional Review Board for the Icelandic Heart Association, and by the Institutional Review Board (IRB) for the US National Institute of Ageing, National Institutes of Health. The Johns Hopkins School of Medicine IRB approved the ancillary study protocol for measurement of serum carboxymethyl-lysine.

Data collection

A standardized protocol was used for fundus photography and is described in detail elsewhere.[27] In brief, after pharmacologic dilation of the pupils, photography was performed in each eye using a 45° 6.3-megapixel digital nonmydriatic camera (Canon, Lake Success, NY). Two photographic fields were taken of each eye, the first centered on the optic disc

and the second centered on the fovea. Software was used for image acquisition and archiving (Eye QSL, Digital Healthcare Inc., Cambridge, United Kingdom). Retinal images were evaluated by the University of Wisconsin Ocular Epidemiology Reading Center for assessment of AMD in a semiquantitative fashion by a grader using EyeQ Lite (an image-processing database for storage, retrieval, and manipulation of digital images) and a standard AMD grading protocol,[28] including the modified Wisconsin Age-related Maculopathy Grading System used in the Multi Ethnic Study of Atherosclerosis.[29] Early AMD required (1) the presence of any soft drusen and pigmentary abnormalities, or (2) the presence of a large soft drusen ≥125 μm in diameter with a large drusen area (>500 μm-diameter circle), or (3) large (≥125 μm in diameter) soft indistinct drusen and no signs of late AMD. Late AMD was defined by the presence of geographic atrophy or exudative AMD. A participant's AMD status was based upon the eye with the more severe disease classification or the eye with gradeable signs if only one eye was graded.

Diagnoses of chronic diseases were made as described elsewhere.[25] The definition of diabetes was based upon self-reported diabetes in the questionnaire and/or use of diabetes medication or hemoglobin A_{1c} ≥6.5%.

Laboratory analyses

Fasting venous blood samples were obtained by brachial venipuncture during the first visit of the 2002-2006 study round. Aliquots of serum were obtained and stored at −80°C. Serum was available for 4709 of the 5272 participants who had fundus images. The measure of circulating AGEs in this study was serum carboxymethyl-lysine (CML), one of the best-characterized AGEs found in the circulation and in tissue.[18,30] CML was measured in a masked fashion using a competitive ELISA (AGE-CML ELISA, Microcoat, Penzberg, Germany). This assay has been validated, is specific, and shows no cross-reactivity with other compounds.[31,32] The inter-assay coefficient of variation (CV) for serum CML was 6%. Serum creatinine was measured using the Jaffe method. Estimated glomerular filtration rate in mL/min/1.73 m^2 was calculated from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation of Levey and colleagues.[33]

Statistical analysis

Continuous and categorical variables were compared across quartiles of serum CML using Kruskal-Wallis tests and chi-square tests, respectively. Univariable and multivariable logistic regression models were used to compare the relationship of serum CML with AMD. Covariates with established associations with AMD such as age, smoking, and BMI were included in the multivariable models. Estimated GFR was included in the final multivariable models because of its known association with circulating CML. All analyses were performed using SAS (v. 9.1.3, SAS Institute, Inc., Cary, NC) with a type I error of 0.05 to determine statistical significance.

Results

The mean (standard deviation [SD]) age was 76.4 (5.5) years for the 4907 participants in the study. The mean (SD) serum CML concentration was 3.0 (0.9) μmol/L. Of the 4907

participants, 1025 (20.9%) had early AMD and 276 (5.6%) had late AMD. The characteristics of the participants are shown by AMD status in Table 1. Participants with early or late AMD were significantly older, had a lower BMI, were current smokers, had higher HDL cholesterol, lower triglycerides, and higher C-reactive protein levels compared with participants who did not have AMD. Participants with early or late AMD were more also significantly more likely to have a history of myocardial infarction and chronic kidney disease. There were no significant differences between participants with and without AMD by sex, alcohol consumption, total cholesterol or LDL cholesterol. The prevalence of hypertension, angina, or diabetes was not associated with AMD status. Higher plasma CML concentrations were weakly associated with AMD ($P = 0.07$).

The characteristics of the participants by quartile of serum CML are shown in Table 2. Greater age, male sex, non-smoking, lower BMI, and higher HDL cholesterol were associated with higher quartiles of serum CML. Higher levels of triglycerides, C-reactive protein, and eGFR were associated with lower quartiles of CML. The prevalence of diabetes was lower and the prevalence of chronic kidney disease was higher among those with higher serum CML. Higher quartiles of CML showed a trend towards with a higher prevalence of hypertension ($P = 0.06$) and lower prevalence of angina ($P = 0.07$). The prevalence of AMD was highest in the top quartile of serum CML. There were no significant associations of total cholesterol, LDL cholesterol, or myocardial infarction with quartiles of serum CML.

Multivariable logistic regression models were used to examine the relationship between serum CML and any AMD (early or late AMD) or late AMD only after controlling for potential confounding (Table 3). In models for the outcome of any AMD (early or late AMD), we observed no significant relationship with log CML (per 1 SD increase) after adjusting for age and sex (Model 1), additionally for BMI and smoking (Model 2), and with the addition of eGFR (Model 3), and finally with addition of diabetes, alcohol consumption, total cholesterol, and HDL cholesterol (Model 4). There was also no significant relationship between the highest versus the lowest quartile of CML in association with any AMD in multivariable models adjusting for the same covariates as above. For late AMD, we observed a suggestion of an association between log CML (per 1 SD increase) when adjusting for age and sex (Model 1, $P = 0.10$), but the relationship was diminished after adjusting for additional covariates, BMI and smoking (Model 2, $P = 0.21$), additionally for eGFR (Model 3, $P = 0.36$), and finally with addition of diabetes, alcohol consumption, total cholesterol, and HDL cholesterol (Model 4, $P = 0.14$). In similar multivariable models comparing the highest quartile of CML versus the lowest quartile, there was no significant relationship between serum CML and late AMD. Alternative multivariable logistic regression models were explored in which either neovascular AMD or geographic atrophy was the dependent variable; serum CML was not significantly associated with either form of late AMD (data not shown).

Discussion

The present study shows that the distribution of circulating CML levels is comparable to that described in other populations and is positively associated with age and inversely associated with kidney function and BMI, as is already known in the scientific literature.[18] Contrary to

our original hypothesis, the study shows that circulating CML is not associated with prevalent AMD in community-dwelling older adults. To our knowledge, this is the first population-based study to examine the relationship between a circulating advanced glycation end product and AMD. The findings from the present study do not corroborate a previous clinic-based study in which mean plasma CML concentrations were >50% higher in 58 cases with AMD (27 with early AMD and 31 with late AMD) compared with 32 controls.[24] In the present study, although mean serum CML concentrations were higher in participants with AMD compared to those without AMD, the difference in circulating CML was minor (~3%) and not statistically significant.

The strengths of the present study are that it involved a large sample size with >1300 cases of AMD, the participants were a population-based sample of community-dwelling adults, AMD was carefully documented using standardized fundus photography and AMD grading at the University of Wisconsin reading center, and that serum CML was measured using a well-characterized assay with low coefficients of variability. Serum CML is the best-characterized circulating AGE in epidemiological studies.[18] The limitations of the study are its cross-sectional design, single measurement of CML, and that only a single type of circulating AGE was measured. Specifically, other AGEs such as pentosidine and hydroimidazolone were not measured, however, previous studies show that circulating CML and pentosidine are moderately correlated.[24] The findings of the present study in a Caucasian population cannot necessarily be extrapolated to other study populations. The relationship between other circulating AGEs and AMD could be explored in future studies.

Elevated circulating CML has been associated with other adverse aging-related outcomes such as cardiovascular and all-cause mortality, arterial stiffness, decline in skeletal muscle strength, and chronic kidney disease.[18] The factors that regulate circulating CML are not clear. Cigarette smoke is a source of AGEs, however, the prevalence of current smoking was actually lower among those with higher serum CML levels. A large population-based study from Finland also found no significant association between higher serum CML levels and smoking.[34] Activation of the AGE-RAGE pathway is thought to increase inflammation,[18] but in the present study, serum CML and C-reactive protein were inversely related. This association was not adjusted for age, sex, or other possible confounders. The lack of an association between serum CML and C-reactive protein is consistent with the Finnish study above.[34] In the present study, although current smoking was higher among those with late AMD, this association was not statistically significant. A review of smoking and AMD showed that 13 of 17 studies showed a statistically significant association between smoking and AMD.[35]

Although it is thought that diabetes contributes to the increased formation of AGES, in the present study, we found no association between plasma CML and diabetes. These findings are consistent with a previous study of glucose metabolism in the Baltimore Longitudinal Study of Aging,[36] a study of patients with type 1 diabetes,[37] and a population-based study of over 800 adults with type 2 diabetes in Finland.[34] In addition, no significant relationship has been found between serum CML and hemoglobin A C,[34,36-38] 1 or between hemoglobin A_1C and low molecular weight AGEs[39] or serum hydroimidazolone.[40]

It has been hypothesized that AGEs contained in food contribute substantially to circulating AGEs.[18] This hypothesis is attractive, since it would suggest that dietary modification may reduce circulating CML levels. The relationship between dietary AGEs and circulating AGEs has not been rigorously studied using stable isotopes. However, recent studies suggest that dietary intake of AGEs does not correlate with either plasma CML concentrations or plasma levels of pentosidine.[41] Another study of 261 adults showed that both serum and urinary CML were not associated with dietary intake of AGEs, as rigorously assessed by six separate 24 hour dietary recalls.[42] Intake of AGE-rich foods was not significantly correlated with serum or urinary CML. Urinary CML was negatively correlated with intake of fast foods.[42]

Although other studies suggest that AGEs in the retina and retinal pigment epithelium play a role in the pathology of AMD, it remains possible that local production and action of AGEs in the eye may participate in the development of AMD. The present study suggests that systemic levels of circulating AGEs are not associated with AMD. Since the present study was cross-sectional, it does not necessarily rule out a role for AGEs in the development of AMD. It is possible that elevated circulating AGEs increase the long-term risk of AMD over time for a subgroup of individuals. Future longitudinal studies are needed to determine whether elevated circulating AGEs are associated with the development or progression of AMD.

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Table 1

Characteristics of 4907 participants, ≥66 years, in the AGES-Reykjavik Study by AMD status

Characteristics[1]		**AMD**			***P***
		None n = 3606	**Early n = 1025**	**Late n = 276**	
Age, y		75.4 (5.2)	78.5 (5.4)	81.4 (5.0)	<0.0001
Sex, % women		56.6	57.1	60.3	0.48
Body mass index, kg/m^2		27.1 (4.4)	26.9 (4.5)	26.3 (4.0)	0.001
Smoking, %	Never/Former	88.1	88.2	84.1	0.12
	Current	11.9	11.8	15.9	
Alcohol, mol/week		0.3 (6.7)	0.3 (7.3)	0.4 (11.2)	0.74
Total cholesterol, mmol/L		5.6 (1.1)	5.6 (1.2)	5.7 (1.1)	0.72
HDL cholesterol, mmol/L		1.6 (0.4)	1.6 (0.5)	1.7 (0.5)	<0.0001
LDL cholesterol, mmol/L		3.5 (1.0)	3.4 (1.0)	3.5 (1.0)	0.61
Triglycerides, mmol/L		1.3 (0.6)	1.1 (0.6)	1.0 (0.6)	<0.0001
C-reactive protein, nmol/L		33.3 (58.0)	36.0 (58.0)	40.9 (71.4)	0.006
CML, µmol/L		3.0 (0.9)	3.1 (1.0)	3.1 (0.9)	0.07
Estimated GFR, mL/min/1.73 m^2		64.5 (15.3)	62.5 (15.2)	61.7 (14.5)	<0.0001
Hypertension, %		80.4	82.3	81.9	0.36
Angina, %		2.4	2.3	2.5	0.98
Myocardial infarction, %		29.1	34.4	40.0	<0.0001
Diabetes, %		11.4	12.4	12.6	0.61
Chronic kidney disease, %		37.2	43.3	46.6	<0.0001

[1] Conversion to SI units as follows: ethanol 1 gm = 0.0217 moles; total cholesterol, HDL cholesterol, LDL cholesterol in mg/dL × 0.0259 = mmol/L; triglycerides mg/dL × 0.0113 = mmol/L; C-reactive protein in mg/L × 9.524 = µmol/L; carboxymethyl-lysine µg/mL × 0.00489 = µmol/L.

Table 2

Characteristics of 4907 participants, ≥66 years, in the AGES-Reykjavik Study by quartile of serum CML

Characteristic[1]		**Quartile of Serum CML, μmol/L**				***P***
		<2.4 n = 1226	**2.4 – 2.9 n = 1228**	**2.9 – 3.5 n = 1227**	**>3.5 n = 1226**	
Age, y		75.1 (5.1)	75.7 (5.3)	76.8 (5.5)	77.8 (5.7)	<0.0001
Sex, % women		59.2	58.5	56.7	52.8	0.007
Body mass index, kg/m^2		28.3 (4.3)	27.3 (4.2)	26.7 (4.2)	26.7 (5.3)	<0.0001
Smoking, %	Never/Former	83.3	86.5	90.1	91.8	<0.0001
	Current	16.7	13.5	9.9	8.2	
Total cholesterol, mmol/L		5.6 (1.1)	5.6 (1.1)	5.6 (1.1)	5.7 (1.1)	0.98
HDL cholesterol, mmol/L		1.5 (0.4)	1.5 (0.4)	1.6 (0.4)	1.7 (0.4)	<0.0001
LDL cholesterol, mmol/L		3.5 (1.0)	3.5 (1.0)	3.5 (1.0)	3.5 (1.0)	0.77
Triglycerides, mmol/L		1.4 (0.6)	1.3 (0.6)	1.1 (0.6)	1.0 (0.6)	<0.0001
C-reactive protein, nmol/L		41.9 (59.0)	34.3 (59.0)	31.4 (59.0)	29.5 (60.0)	<0.0001
Estimated GFR, mL/min/1.73 m^2		66.1 (14.5)	65.2 (14.5)	63.3 (14.5)	61.1 (14.6)	<0.0001
AMD, %	No disease	71.9	71.6	76.1	74.3	0.03
	Early or late disease	28.1	28.4	23.9	25.7	
AMD, %	No or early disease	94.4	93.7	94.6	94.8	0.64
	Late disease	5.6	6.3	5.4	5.2	
Hypertension, %		80.9	79.8	80.8	81.9	0.06
Angina, %		3.3	2.6	1.6	1.9	0.07
Myocardial infarction, %		33.8	29.3	30.4	29.6	0.15
Diabetes, %		16.0	12.8	8.3	9.6	<0.0001
Chronic kidney disease, %		33.4	36.2	41.2	45.1	<0.0001

[1] All variables from body mass index through chronic kidney disease were adjusted for age and sex.

Table 3

Multivariable logistic regression models for serum CML and any AMD (early or late) or late AMD only

		Model 1 Adjusted for age, sex			Model 2 Adjusted for age, sex, BMI, smoking			Model 3 Adjusted for age, sex, BMI, smoking, eGFR			Model 4 Adjusted for age, sex, BMI, smoking, eGFR, diabetes, alcohol, total cholesterol, HDL cholesterol		
		OR	**95% CI**	***P***	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Any AMD (early or late)	Log CML, per 1 SD increase	0.94	0.88, 1.01	0.07	0.96	0.90, 1.03	0.27	0.97	0.90, 1.04	0.44	1.06	0.99, 1.15	0.09
	Highest vs lowest quartile of CML	0.87	0.72, 1.05	0.40	0.91	0.75, 1.11	0.70	0.94	0.77, 1.14	0.88	0.84	0.69, 1.03	0.30
Late AMD only	Log CML, per 1 SD increase	0.90	1.02, 2.70	0.10	0.92	0.80, 1.05	0.21	0.94	0.82, 1.08	0.36	1.11	0.97, 1.27	0.14
	Highest vs lowest quartile of CML	1.16	0.96, 1.40	0.31	1.10	0.91, 1.33	0.58	1.07	0.88, 1.30	0.78	0.91	0.61, 1.34	0.47