ORIGINAL INVESTIGATION | EPIDEMIOLOGY

Serum Carboxymethyllysine, an Advanced Glycation End Product, and Age-Related Macular Degeneration
The Age, Gene/Environment Susceptibility–Reykjavik Study

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

OBJECTIVE To investigate the relationship between serum carboxymethyllysine (CML), a major circulating advanced glycation end product, and AMD in older adults.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study of a population-based sample of 4907 older adults (aged ≥66 years) in the Age, Gene/Environment Susceptibility–Reykjavik Study in Iceland.

EXPOSURES Serum CML and risk factors for AMD.

MAIN OUTCOMES AND MEASURES Early or late AMD, assessed through fundus images taken through dilated pupils using a 45° 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.

RESULTS Of the 4907 participants, 1025 (20.9%) had early AMD and 276 (5.6%) had late AMD. Mean (SD) serum CML concentrations among adults with no AMD, early AMD, and late AMD (exudative AMD and pure geographic atrophy) were 618.8 (195.5), 634.2 (206.4), and 638.4 (192.0) ng/mL, respectively (to convert to micromoles per liter, multiply by 0.00489; \( P = .07 \)). Log serum CML (per 1-SD increase) was not associated with any AMD (early and late AMD) (odds ratio = 0.97; 95% CI, 0.90-1.04; \( P = .44 \)) or with late AMD (odds ratio = 0.94; 95% CI, 0.82-1.08; \( P = .36 \)) in respective multivariable logistic regression models adjusting for age, sex, body mass index, smoking, and renal function.

CONCLUSIONS AND RELEVANCE Higher serum CML concentration had no significant cross-sectional association with prevalent AMD in this large population-based cohort of older adults in Iceland.
Age-related macular degeneration (AMD) is the leading cause of vision loss among adults aged 65 years and older in developed countries. 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 United States alone. The global cost of visual impairment due to AMD alone was estimated at $343 billion in 2010, including $255 billion in direct health care costs. Lifestyle and dietary modifications, intravitreal antiangiogenic therapy, and antioxidant supplementation are among the current strategies to reduce the morbidity of AMD. 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 or innate immune dysregulation as well as oxidative stress. Age is a strong risk factor for AMD, with the prevalence of advanced AMD increasing from about 0.2% in ages 55 to 64 years to 13% in those older than 85 years. Smoking, obesity, white race, and low intake of dietary antioxidants and ω-3 fatty acids are associated with an increased risk of AMD. There is a strong genetic susceptibility to AMD as shown in twin studies, familial aggregation analyses, 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. 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 as well as extracellular matrix genes such as hemicentin 1 and fibulin 5 in AMD risk. Although variants within identified major susceptibility genes to AMD play a role in more than half of AMD cases, many individuals carrying AMD risk genotypes never develop the disease, and only a fraction diagnosed as having it progress to advanced AMD with vision loss.

Advanced glycation end products (AGEs) are a heterogeneous group of bioactive molecules formed by the nonenzymatic glycation of proteins, lipids, and nucleic acids. They are implicated in a wide number of adverse age-related outcomes, including cardiovascular disease, diabetes mellitus, chronic kidney disease, osteoporosis, and sarcopenia. They alter the structural integrity of tissues by cross-linking collagen and are thought to upregulate inflammation through binding with the receptor for AGEs (RAGE). The 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, carboxymethyllysine (CML) in drusen of eyes with AMD, and AGEs and RAGE in photoreceptors and retinal pigment epithelium of eyes with AMD. Basal laminar deposits, which develop between the retinal pigment epithelial cells and the basement membrane and are specific for AMD, show greatly increased expression of RAGE. A key factor in the pathogenesis of neovascular AMD is the expression of vascular endothelial growth factor. The activation of RAGE leads to the increased expression of vascular endothelial growth factor via the activation of NF-κB. One study suggested that plasma CML and pentosidine concentrations were higher in 58 patients with AMD compared with 32 control participants, but further corroboration is needed.

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

Methods

Study Participants

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 (including systemic disease as well as eye disease) in older people. The study design and assessment of the cohort have been described elsewhere. 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 5764 individuals were examined for the AGES-Reykjavik Study in 2002 to 2006. The comprehensive AGES-Reykjavik Study protocol required each participant to complete 3 visits to the Icelandic Heart Association Research Center within 3 to 6 months. The ocular component was included as part of the third visit in which 5330 persons participated. As part of the assessments at the Icelandic Heart Association Research Center, a questionnaire was administered, visual acuity was assessed, and images were acquired from the retina. Fundus images were available from 5272 individuals for the determination of AMD status. Written informed consent was obtained from all participants. The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee, which acts as the institutional review board for the Icelandic Heart Association, and by the institutional review board for the US National Institute on Aging, National Institutes of Health. The Johns Hopkins School of Medicine institutional review board approved the ancillary study protocol for measurement of serum CML concentration.

Data Collection

A standardized protocol was used for fundus photography and is described in detail elsewhere. In brief, after pharmacologic dilation of the pupils, photography was performed in each eye using a 45° 6.3-megapixel digital nonmydriatic camera (Canon). Two photographic fields were taken of each eye, with 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). 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, including the modified Wisconsin Age-Related Maculopathy Grading System used in the Multi-Ethnic Study of Atherosclerosis. Early
Serum Carboxymethyllysine and AMD

Table 1. Characteristics of 4907 Participants Aged 66 Years or Older in the Age, Gene/Environment Susceptibility–Reykjavik Study by Age-Related Macular Degeneration Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>None (n = 3606)</th>
<th>Early (n = 1025)</th>
<th>Late (n = 276)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>75.4 (5.2)</td>
<td>78.5 (5.4)</td>
<td>81.4 (5.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female, %</td>
<td>56.6</td>
<td>57.1</td>
<td>60.3</td>
<td>.48</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>27.1 (4.4)</td>
<td>26.9 (4.5)</td>
<td>26.3 (4.0)</td>
<td>.001</td>
</tr>
<tr>
<td>Smoking, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never or former</td>
<td>88.1</td>
<td>88.2</td>
<td>84.1</td>
<td>.12</td>
</tr>
<tr>
<td>Current</td>
<td>11.9</td>
<td>11.8</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption, mean (SD), g/wk</td>
<td>13.8 (308.8)</td>
<td>13.8 (336.4)</td>
<td>18.4 (516.1)</td>
<td>.74</td>
</tr>
<tr>
<td>Cholesterol, mean (SD), mg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>216.2 (42.5)</td>
<td>216.2 (46.3)</td>
<td>220.1 (42.5)</td>
<td>.72</td>
</tr>
<tr>
<td>HDL</td>
<td>61.8 (15.4)</td>
<td>61.8 (19.3)</td>
<td>65.6 (19.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL</td>
<td>135.1 (38.6)</td>
<td>131.3 (38.6)</td>
<td>135.1 (38.6)</td>
<td>.61</td>
</tr>
<tr>
<td>Triglycerides, mean (SD), mg/dL</td>
<td>115.0 (53.1)</td>
<td>97.3 (53.1)</td>
<td>88.5 (53.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C-reactive protein, mean (SD), mg/L</td>
<td>3.5 (6.1)</td>
<td>3.8 (6.1)</td>
<td>4.3 (7.5)</td>
<td>.006</td>
</tr>
<tr>
<td>CML, mean (SD), ng/mL</td>
<td>618.8 (195.5)</td>
<td>634.2 (206.4)</td>
<td>638.4 (192.0)</td>
<td>.07</td>
</tr>
<tr>
<td>eGFR, mean (SD), mL/min/1.73 m²</td>
<td>64.5 (15.3)</td>
<td>62.5 (15.2)</td>
<td>61.7 (14.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>80.4</td>
<td>82.3</td>
<td>81.9</td>
<td>.36</td>
</tr>
<tr>
<td>Angina, %</td>
<td>2.4</td>
<td>2.3</td>
<td>2.5</td>
<td>.98</td>
</tr>
<tr>
<td>Myocardial infarction, %</td>
<td>29.1</td>
<td>34.4</td>
<td>40.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>11.4</td>
<td>12.4</td>
<td>12.6</td>
<td>.61</td>
</tr>
<tr>
<td>Chronic kidney disease, %</td>
<td>37.2</td>
<td>43.3</td>
<td>46.6</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CML, carboxymethyllysine; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Statistical Analysis
Continuous and categorical variables were compared across quartiles of serum CML concentration using Kruskal-Wallis tests and χ² tests, respectively. Univariable and multivariable logistic regression models were used to compare the relationship of serum CML level with AMD. Covariates with established associations with AMD such as age, smoking, and body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) were included in the multivariable models. The eGFR was included in the final multivariable models because of its known association with circulating CML. All analyses were performed using SAS version 9.1.3 statistical software (SAS Institute, Inc) with a type I error of 0.05 to determine statistical significance.

Results
The mean (SD) age was 76.4 (5.5) years for the 4907 participants in the study. The mean (SD) serum CML concentration was 623.1 (197.7) ng/mL (to convert to micromoles per liter, multiply by 0.00489). 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 high-density lipoprotein cholesterol and C-reactive protein levels, and had
lower triglycerides levels compared with participants without AMD. Participants with early or late AMD were 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 level, or low-density lipoprotein cholesterol level. The prevalences of hypertension, angina, and diabetes were not associated with AMD status. Higher plasma CML concentrations were weakly associated with AMD ($P = .07$).

The characteristics of the participants by quartile of serum CML concentration are shown in Table 2. Being older, being male, not smoking, having a lower BMI, and having a higher HDL cholesterol level were associated with higher quartiles of serum CML concentration. Higher triglycerides level, C-reactive protein level, and eGFR were associated with lower quartiles of CML concentration. The prevalence of diabetes was lower and the prevalence of chronic kidney disease was higher among those with higher serum CML concentration. Higher quartiles of CML concentration showed a trend toward a higher prevalence of hypertension ($P = .06$) and a lower prevalence of angina ($P = .07$). The prevalence of AMD was highest in the top quartile of serum CML concentration. There were no significant associations of total cholesterol level, low-density lipoprotein cholesterol level, or myocardial infarction with quartiles of serum CML concentration.

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 adjusting for BMI and smoking (model 2), with the addition of eGFR (model 3), and finally with addition of diabetes, alcohol consumption, total cholesterol level, and HDL cholesterol level (model 4). There was also no significant relationship between the highest vs lowest quartiles of CML concentration in association with any AMD in multivariable models adjusting for the same covariates as described earlier. 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, $P = .14$), but the relationship diminished after adjusting for BMI and smoking (model 2, $P = .21$), additionally for eGFR (model 3, $P = .36$), and finally with the addition of diabetes, alcohol consumption, total cholesterol level, and HDL cholesterol level (model 4, $P = .14$). In similar multivariable models comparing the highest quartile of CML concentration vs the lowest quartile, there was no significant relationship between serum CML concentration and late AMD. Alternative multivariable logistic regression models were explored in which either neovascular AMD or geographic atrophy was the dependent variable; serum CML concentration was not significantly associated with either form of late AMD (data not shown).
Table 3. Multivariable Logistic Regression Models for Serum Carboxymethyllysine Concentration and Any (Early or Late) or Late Age-Related Macular Degeneration*

<table>
<thead>
<tr>
<th>AMD Status and CML Concentration</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td>Any AMD, early or late</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log CML, per 1-SD increase</td>
<td>0.94 (0.88-1.01)</td>
<td>.07</td>
<td>0.96 (0.90-1.03)</td>
<td>.27</td>
</tr>
<tr>
<td>Highest vs lowest quartile of CML</td>
<td>0.87 (0.72-1.05)</td>
<td>.40</td>
<td>0.91 (0.75-1.11)</td>
<td>.70</td>
</tr>
<tr>
<td>Late AMD only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log CML, per 1-SD increase</td>
<td>0.90 (1.02-2.70)</td>
<td>.10</td>
<td>0.92 (0.80-1.05)</td>
<td>.21</td>
</tr>
<tr>
<td>Highest vs lowest quartile of CML</td>
<td>1.16 (0.96-1.40)</td>
<td>.31</td>
<td>1.10 (0.91-1.33)</td>
<td>.58</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; CML, carboxymethyllysine; OR, odds ratio.
* Model 1 adjusted for age and sex; model 2, age, sex, body mass index (BMI); calculated as weight in kilograms divided by height in meters squared), and smoking; model 3, age, sex, BMI, smoking, and estimated glomerular filtration rate; and model 4, age, sex, BMI, smoking, estimated glomerular filtration rate, diabetes mellitus, alcohol consumption, total cholesterol, and high-density lipoprotein cholesterol.

Discussion

This study shows that the distribution of circulating CML levels is comparable to that described in other populations; it is positively associated with age and inversely associated with kidney function and BMI, as is already shown in the literature.34 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 AGE and AMD. The findings from this study do not corroborate a previous clinic-based study in which mean plasma CML concentrations were higher in those with AMD compared with those without AMD; the difference in circulating CML concentration was minor (approximately 3%) and not statistically significant.

The strengths of our study are that it involved a large sample with more than 1300 cases of AMD, the participants were a population-based sample of community-dwelling adults, AMD was carefully documented using standardized fundus photographs and AMD grading at the University of Wisconsin Ocular Epidemiology Reading Center, and 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 measurement of only a single type of circulating AGE. 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 this study in a white population cannot necessarily be extrapolated to other study populations. The relationship between other circulating AGEs and AMD could be explored in future studies.

Elevated concentration of circulating CML has been associated with other adverse age-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, but 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,38 but in our study, serum CML and C-reactive protein levels 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 levels is consistent with the Finnish study.34 In our study, although current smoking was higher among those with late AMD, this association was not statistically significant. A review of smoking and AMD indicated 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 our study we found no association between plasma CML level and diabetes. These findings are consistent with a previous study of glucose metabolism in the Baltimore Longitudinal Study of Aging,26 a study of patients with type 1 diabetes,37 and a population-based study of more than 800 adults with type 2 diabetes in Finland.34 In addition, no significant relationship has been found between serum CML and hemoglobin A1c levels34-36 or between levels of hemoglobin A1c and low-molecular-weight AGEs39 or serum hydroimidazolone.40

It has been hypothesized that AGEs contained in food contribute substantially to circulating AGEs.18 This hypothesis is attractive because 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 pentosidine concentrations.41 Another study of 261 adults showed that both serum and urinary CML concentrations were not associated with dietary intake of AGEs, as rigorously assessed by 6 separate 24-hour dietary recalls.42 Intake of AGE-rich foods was not significantly
Conclusions

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. Our study suggests that systemic levels of circulating AGEs are not associated with AMD. Because this study was cross-sectional, it does not necessarily exclude a role for AGEs in the development of AMD. It is possible that elevated concentrations of 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 levels of circulating AGEs are associated with the development or progression of AMD.

REFERENCES


