Original Research Article

N-carboxymethyllysine as a biomarker for coronary artery disease and age-related macular degeneration

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ABSTRACT

Background and objective: An association between coronary artery disease (CAD) and age-related macular degeneration (ARMD) has long been postulated, but exact mechanisms remain unclear. The global prevalence of CAD and ARMD increases and early biomarkers for early diagnosis of these diseases are necessary. The aim of this study was to investigate the plasma level of oxidative stress biomarker CML in patients with and without angiographic findings of atherosclerosis in the coronary arteries (CADath+ and CADath−, respectively) and to assess if there was an association of CAD with ARMD.

Materials and methods: The study enrolled 233 subjects. Based on cardiologic and ophthalmologic examinations, the patients were divided into four subgroups: CADath+ARMD+, CADath+ARMD−, CADath−ARMD+, and CADath−ARMD−. The enzyme-linked immunosorbent assay was used for the measurement of plasma CML levels. Serum lipid levels were determined by an automatic analyzer using conventional enzymatic methods.

Results: CADath+ patients had higher CML concentration compared to CADath− subjects (1.04 ± 0.6 vs. 0.83 ± 0.4 ng/mL, $P < 0.001$). The highest mean CML level (1.12 ± 0.7 ng/mL) was found in CADath+ARMD+ patients. The mean plasma CML concentration was higher in subjects with any of the analyzed diseases compared to CADath−ARMD− subjects. A significant positive association of CADath+ (OR = 2.50, 95% CI 1.60–3.90, $P = 0.0001$), ARMD (OR = 2.08, 95% CI 1.40–3.11, $P = 0.0001$) and both analyzed diseases (OR = 4.67, 95% CI 2.29–9.53, $P = 0.0001$) with an increased level of plasma CML in a logistic regression model adjusting by age was identified.

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Conclusions: The level of CML, an oxidative stress biomarker, reflects the presence of atherosclerosis in coronary arteries and shows a possible link between ARMD and CADath+ via oxidative status.

1. Introduction

Coronary artery disease (CAD) is the leading cause of death worldwide in both men and women [1], and age-related macular degeneration (ARMD) is known to be the major cause of uncorrectable visual impairment in the elderly population of developed countries [2]. ARMD risk factors are in close connection, correlate with, and often are identical to the risk factors of cardiovascular diseases [3]. There is some evidence that both these diseases may share common pathogenesis: morphological and immunohistological changes in ARMD are similar to those in the arterial intima in cases with atherosclerosis [4]. In recent studies atherosclerosis is defined as local arterial intima inflammation, caused by the interplay between lipid metabolism and oxidative stress [5,6].

N-carboxymethyllysine (CML) – a product of both lipoxidation and glycoxidation [7] – represents a general marker of oxidative stress and long-term damage to proteins [8]. In vivo studies revealed strong intracellular CML staining areas of histiocytic/monocytic infiltration or proliferation, mostly associated with atheroma formation [9]. Given that multivessel flow-limiting obstructions are observed in patients with chronic coronary syndrome, atheroma formation and, consequently, atherosclerotic plaques seem to affect coronary flow [10]. Furthermore, CML accumulation detected locally in the eyes of patients with ARMD [11–13] suggests that similar oxidative stress may also show up in the retina, where it can result in tissue damage, and may lead to irreversible central vision loss. It has been stated that relative ischemia of the outer retina that may be caused by atherosclerosis and atrophy of the choriocapillaris is involved in the development of exudative ARMD [14].

Recent studies [2,4–6,15] aiming at preventing the development of CAD and ARMD are still striving to identify the underlying pathomechanisms of both common disorders. We aimed to investigate the plasma levels of CML in patients with and without angiographic findings of atherosclerosis in the coronary arteries; moreover, an attempt was made to assess the potential association of atherosclerosis in coronary arteries with ARMD.

2. Materials and methods

2.1. Study population

The study was performed with the permission No. BE-2-28 issued by the Kaunas Regional Biomedical Research Ethics Committee. A written informed consent was obtained from all participants prior to their inclusion into the study.

The recent case-control study enrolled 233 patients who underwent a detailed cardiologic examination including diagnostic coronary angiography at the Department of Cardiology, Hospital of the Lithuanian University of Health Sciences (LUHS). The inclusion criterion was age ≥45 years for both sexes. Exclusion criteria of the study included diabetes mellitus, serious systemic diseases (somatic illness like oncological or mental disorders); retinal diseases other than ARMD, ocular trauma in the past; and refusal to participate.

The participants were divided into two groups as follows: the CADath− group included the subjects without angiographic findings of atherosclerosis in the coronary arteries and the CADath+ group, the patients with atherosclerotic lesions in the coronary arteries.

CADath− was defined by the absence of any lesion with a diameter of stenosis less than 50% on quantitative coronary angiography of the baseline coronary angiogram. CADath+ was defined as stenosis greater than or equal to 50% of the vessel lumen in one, two, or three main arteries.

All the participants were examined by an ophthalmologist at the Department of Ophthalmology of the LUHS to confirm or rule out ARMD. The ophthalmological examination included best-corrected visual acuity recording using manifest refraction, and the LogMar visual acuity chart, Schiøtz tonometry, slit lamp-assisted biomicroscopy of the anterior and the posterior segments of the eye, and stereoscopic fundus photographs (Carl Zeiss Meditec AG, Germany). Optical coherence tomography (Zeiss Stratus OCT 3000) and fluorescence angiography were performed in patients with suspected exudative ARMD. The diagnosis of ARMD was made if confirmed by two ophthalmologists, and if no other retinal disorders were found during a detailed ophthalmological examination. Finally, the patients were divided into four subgroups: CADath+ARMD+ ($n = 77$), CADath+ARMD− ($n = 67$), CADath−ARMD+ ($n = 47$), and CADath−ARMD− ($n = 42$).

The eye examination was performed and blood samples were taken 3–6 months after coronary angiography. The subjects were asked to abstain from food and drinks for 12–14 h before blood sampling. Total serum cholesterol (TChol), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides (TG), and plasma CML concentration were measured.

2.2. Laboratory analyses and anthropometric measurements

Blood samples for measurement of CML were collected in vacuum tubes using EDTA as an anticoagulant (EDTA-K3). Plasma samples were prepared within 50–70 min and then stored at −70 °C until analysis. Frozen plasma samples storage time averaged 60 days and thawed only once.