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Featured Article

Serum concentrations of vitamin E and carotenoids are altered in Alzheimer's disease: A case-control study

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Abstract

Introduction: Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease (AD). We investigated associations between serum levels of lipophilic antioxidants and AD.

Methods: Serum concentrations of retinol, two forms of vitamin E (α- and γ-tocopherol) and six carotenoids were quantified by high-performance liquid chromatography from patients with AD ($n = 251$) and cognitively intact controls ($n = 308$) and assessed by regression analyses.

Results: Serum levels of α-tocopherol and all six carotenoids were significantly lower in patients with AD compared with cognitively intact controls ($P < .001$). In contrast, γ-tocopherol was significantly higher in the serum of patients with AD (odds ratio = 1.17 [confidence intervals: 1.05–1.31]).

Discussion: Our findings implicate compromised serum antioxidant defenses in AD pathogenesis and differing biological roles for vitamin E isoforms. This highlights the need for improved understanding in the balanced upregulation of exogenous antioxidants related to dietary intake or supplement use in future nutritional intervention studies.

Keywords: α-Carotene; α-Tocopherol; β-Carotene; β-Cryptoxanthin; γ-Tocopherol; Lutein; Lycopene; Retinol; Zeaxanthin

1. Introduction

Alzheimer's disease (AD) is the most common dementia subtype accounting for approximately 75% of all cases [1]. Multiple neuropathological processes underlie the onset of cognitive decline leading to disease, a process likely influenced by a range of both modifiable and nonmodifiable risk factors [2].

Among the etiological processes proposed, there is increasing support for oxidative injury and inflammatory damage early in AD pathogenesis [3–5]. The brain is especially vulnerable to reactive oxygen species due to neurons possessing relatively low levels of endogenous antioxidants to cope with their high metabolic activity. This antioxidant deficit results in oxidative damage to major cell components with elevated levels of inflammatory markers resulting in neuronal cell death [4].

Nutritional influences in AD may provide significant public health benefit, but the influence of dietary antioxidants on AD risk remains unclear. Plausible mechanisms by which nutritional factors such as serum carotenoids and vitamins may reduce cognitive decline have been proposed, such as antioxidative and/or anti-inflammatory processes, including those common to vascular and nonvascular diseases [6].

Despite numerous studies examining a variety of antioxidants, the most recent systematic review of serum antioxidant status in AD reported the overall quality of evidence to be low, due to insufficient control for potential confounders in studies with low power producing inconsistent findings, and also due to the relative absence of randomized control trials [7]. We sought to address these limitations through increased sample size with adjustment of

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effect estimates for potential confounding variables. We compared serum retinol, α-tocopherol, and γ-tocopherol (referred to as antioxidant vitamins) as well as the six major carotenoids found in considerable concentrations in serum: lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene in patients with AD and cognitively intact controls. We sought to evaluate any associations between levels of exogenous antioxidants in sera of participants with AD risk.

2. Methods

2.1. Study population

This was a prevalent case-control study in which cases with AD were compared to cognitively intact controls. All recruitment and testing were performed by one investigator (M.A.W.) from August 2006 to 2008 and has been described elsewhere [8]. Methods reported were guided as far as possible by STROBE guidelines for case-control studies [9]. Cases and controls were not matched. The power calculation for sample size was based on a genetic outcome of interest, reported elsewhere [10]. Potential AD cases were identified as they appeared in the memory clinic in Belfast City Hospital, UK and Knockbracken Healthcare Park, UK or from records of previous attendees in the same clinics. AD was defined as per the diagnosis of a senior clinician using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS ADRDA) criteria [11]. Exclusion criteria were being diagnosed with other types of dementia, including vascular or mixed dementia, applied to ensure as far as feasible the cases consisted of AD cases only. The carer was approached in person or by phone, an opportunity given for the carer and patient to ask questions and permission sought to contact them again. After at least 24 hours to read the information sheet, carers were contacted, and if they and the person with AD were willing to participate, the study visit was arranged.

The recruitment strategies used in the enrollment of controls were designed to identify enough cognitively normal individuals in a practical manner. First, carers of patients attending any outpatient clinic in the study hospitals were approached. Second, a university press release invited participation in the study. Third, a series of talks given to AD patient-support groups in the region led to further volunteers coming forward. Fourth, controls asked their friends and relatives to participate. Exclusion criteria for controls included age under 65 years, to mirror if not formally match cases' ages, a Mini–Mental State Examination (MMSE) score of below 26 of 30 to try to exclude undiagnosed AD cases from being controls, or any history of neurological disease or dementia. After the study visit, there was no follow-up for cases or controls.

Ethics and clinical governance approval was obtained before commencement of the study which adhered to the tenets of the Declaration of Helsinki. On enrollment, all study participants underwent an assessment that involved drawing a blood sample, measuring blood pressure, and performing a MMSE. The final component of the assessment involved the completion of questionnaires via interviews with the subject, as well as their carer when appropriate.

2.2. Detection of serum dietary antioxidants

Serum samples were coded and stored at −80°C for extraction and batch analysis in a blinded fashion. Serum concentrations of retinol, α-tocopherol, γ-tocopherol, and six carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, zeaxanthin) were determined by high-performance liquid chromatography (HPLC) with diode array detection [12].

Chromatograms were analyzed using ChromQuest 4.2 software (Thermo Fisher Scientific, MA, USA). The mobile phase consisted of 97% methanol/3% tetrahydrofuran with solvents degassed using an in-line degasser before the HPLC pump. The flow rate was 1 mL/minute and the column was maintained at 31°C. The HPLC components were all ThermoSeparation Products allowing for the simultaneous detection of three wavelengths of magnitude 325, 292, and 450 nm.

All analytes were quantified using external standards (Sigma-Aldrich, Poole, Dorset, UK and Chemos GmbH, Regensburg, Germany) with validation against the National Institute of Standards and Technology standard reference material 968d for fat-soluble vitamins, carotenoids, and cholesterol in human serum. In-house quality control samples were also included in every run. The inter-assay and intra-assay coefficients of variation for vitamin A, E, and the carotenoid assay were both <15% [12].

2.3. Other variables

DNA extracted from the blood sample drawn enabled *APOE* genotyping of participants by "Sequenom iPLEX assay." A family history of AD and all comorbid health conditions were documented as present or absent as determined by self-report or consultation of medical notes. Smoking history was measured as a cumulative dose in pack-years.

2.4. Statistical analysis

Summary statistics for continuous variables and frequencies and relative frequencies by group were calculated. Independent t-tests (for continuous variables) or chi-square tests (for categorical variables) were used to compare participant characteristics between cases and controls. Pearson's correlation coefficients were performed to identify associations between cognitive indices with age and other continuous variables.