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Blood-Based Biomarkers

Plasma sphingolipid changes with autopsy-confirmed Lewy body or Alzheimer's pathology

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Abstract

Introduction: The clinical and pathological phenotypes of Dementia with Lewy bodies (DLB) and Alzheimer's disease (AD) often overlap. We examined whether plasma lipids differed among individuals with autopsy-confirmed Lewy Body pathology or AD pathology.

Methods: We identified four groups with available plasma 2 years before death: high (n = 12) and intermediate-likelihood DLB (n = 14) based on the third report of the DLB consortium; dementia with Alzheimer's pathology (AD; n = 18); and cognitively normal with normal aging pathology (n = 21). Lipids were measured using ESI/MS/MS.

Results: There were overall group differences in plasma ceramides C16:0, C18:1, C20:0, and C24:1 and monohexosylceramides C18:1 and C24:1. These lipids did not differ between the high-likelihood DLB and AD groups, but both groups had higher levels than normals. Plasma fatty acid levels did not differ by group.

Discussion: Plasma ceramides and monohexosylceramides are elevated in people with dementia with either high-likelihood DLB or AD pathology.

Keywords: Alzheimer's disease; Lewy body; Autopsy; Lipids; Ceramide

1. Introduction

Alzheimer's disease (AD) dementia and dementia with Lewy bodies (DLB) are two of the most common dementias in the population [1]. The clinical and pathologic phenotypes of AD and DLB are heterogeneous and often overlap [2], so there is a critical need to identify biomarkers to aid in the differential diagnosis of DLB and AD. Given the high degree of clinical heterogeneity of DLB, the use of neuropathologically confirmed cases is essential for identifying potential DLB-specific biomarkers.

Lipids directly affect the solubility and fluidity of cell membranes. The homeostasis of membrane and intracellular lipids in neuron and myelin is a key component in preventing loss of synaptic plasticity, cell death, and ultimately, neurodegeneration [3]. In addition to structural roles, many sphingolipids are also bioactive and involved in signaling pathways. Both alpha-synuclein, the primary constituent of Lewy Bodies (LB), and amyloid-beta, the primary constituent of amyloid

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plaques, are involved in the regulation of membrane lipid composition and can also be modified by specific lipids [4,5].

Experimental evidence suggests that mutations in the *GBA* gene coding for glucocerebrosidase, which catalyzes the conversion of glucosylceramide (a monohexosylceramide) to ceramide and glucose, cause a build-up of lysosomal glucosylceramide. This accumulation may promote the oligomerization of alpha-synuclein, the decreased degradation of lysosomal alpha-synuclein and ultimately subsequent neurodegeneration [6]. Mutations in *GBA* are the most prevalent risk factor for sporadic DLB [7,8]. Furthermore, plasma ceramide and monohexosylceramide levels were found to be elevated in sporadic, non-*GBA* Parkinson's disease patients, with the and highest levels among cognitively impaired patients [9].

In addition to an association between specific sphingolipids and DLB, several lines of evidence suggest both direct and indirect associations between ceramides and amyloid-beta (Aβ) levels, the hallmark AD pathology [10–15]. Using a sample of clinically diagnosed cases, we found that plasma ceramide levels predicted risk of cognitive impairment and AD among cognitively normal individuals [16,17], memory decline and hippocampal volume loss among patients with amnestic mild cognitive impairment [18], and faster rates of cognitive decline among AD patients [19]. The primary objective of the present study was to determine whether levels of ceramides and monohexosylceramides, measured at the last visit before autopsy, could be useful blood-based biomarkers indicative of LB and/or AD pathology. We assayed a panel of fatty acids to assess the specificity of any findings to sphingolipids as opposed to global lipid changes in these neurodegenerative disorders. A secondary objective was to determine whether there was a relationship between the plasma lipids and amyloid and neurofibrillary tangle pathology.

2. Methods

All individuals were enrolled in the Mayo Clinic Alzheimer's Disease Research Center (ADRC) and donated their brain to the Neuropathology Core. Eligibility criteria for the proposed study included an autopsy report and available blood at the last study visit before death. Standardized methods for sampling and neuropathologic examination were performed according to the third report of the DLB consortium (CDLB) [20,21] and the Consortium to Establish a Registry for Alzheimer's Disease guidelines [22]. Braak neurofibrillary tangle (NFT) stage was determined based on the distribution of NFTs assessed with Bielschowsky silver stain [23]. A consensus clinical diagnosis was determined at each study visit by a panel of neurologists, neuropsychologists, and nurses who reviewed all patient information including neuropsychological results, activities of daily living, and the Clinical Dementia Rating scale. The diagnosis of dementia was based on DSM-III-R criteria [24]. We identified the following four groups: (1) Cognitively normal-normal pathology [CN, n = 21]. These individuals had no LBs, had low-likelihood AD according to the National Institute of Aging (NIA)-Reagan Criteria [25], and were cognitively normal as of their last study visit. (2) High-likelihood DLB [n = 13]. These individuals met criteria for high-likelihood DLB according to the CDLB, had Braak NFT stage $\leq$ IV, low to intermediate-likelihood AD, and a diagnosis of dementia as of the last study visit. Twelve patients had diffuse LB and one had transitional LB. (3) Intermediate-likelihood DLB (n = 17). These individuals had both DLB and AD pathologies. They had transitional (n = 14) or diffuse (n = 3) LBs, met criteria for intermediate-likelihood DLB according to the CDLB, had Braak NFT stage $\geq$ IV, intermediate to high-likelihood AD, and a diagnosis of dementia as of the last study visit. (4) Alzheimer's disease pathology (AD, n = 18). These individuals had high (n = 16) or intermediate (n = 2) likelihood AD according to NIA-Reagan criteria, had Braak NFT stage $\geq$ IV, no LBs, and had a diagnosis of probable AD dementia. Given our previous report that plasma ceramides increase with age and are higher in women [26], we frequency matched the four groups by sex and also by age.

2.1. Blood collection procedures

All blood samples in the Mayo Clinic ADRC are collected from nonfasting participants in the sitting position in a clinical laboratory. Serum tubes (red-top) are drawn first, followed by EDTA plasma tubes. Blood is drawn from the median cubital vein with a 21 g needle and typically centrifuged at 2000 g for 10 minutes at 4°C. The serum and plasma are aliquoted into 0.5 mL and stored in a −80°C freezer until use. None of the aliquots were thawed before being pulled to measure the sphingolipids and fatty acids. We have previously shown that long-term storage, up to 38 years, in long-term −80°C freezers does not affect sphingolipid levels [26].

2.2. Assay methodology

The targeted sphingolipid and fatty acid analyses were conducted by the Mayo Clinic Metabolomics Core. Electrospray ionization mass spectrometry was used to quantify plasma ceramides, sphinganine, sphingosine, sphingosine-1-phosphate (S1P), monohexosylceramides, and free fatty acids. The lipids were extracted from 200 μL of plasma after the addition of internal standards. The extracts were measured against a standard curve on the Thermo TSQ Quantum Ultra mass spectrometer (West Palm Beach, FL) coupled with a Waters Acquity UPLC system (Milford, MA) and quantified in μM units.

2.3. Statistical analyses

Our primary analyses focused on identifying group differences in the demographic and clinical variables and in plasma lipids using Kruskal–Wallis rank tests. In subsequent analyses, we also examined the association between the plasma lipids and NIA-Reagan Criteria and Braak NFT