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The role of human kallikrein 6, clusterin and adiponectin as potential blood biomarkers of dementia

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ABSTRACT

Objectives: Progressive degenerative syndromes which affect brain, altering memory, behavior, cognition and emotion, are commonly defined as dementia. It was suggested that serum human kallikrein 6 (KLK6), clusterin (CLU) and adiponectin (ADPN) in combination with inflammation markers, neuroimaging and neuropsychological testing could assist in discriminating dementia patients from control individuals. Our aim was therefore to compare serum concentrations of KLK6, CLU and ADPN and inflammatory marker, interleukin-6 (IL-6), in patients suffering from Alzheimer's disease (AD), patients with vascular dementia (VAD), cognitively healthy participants (CHP) and those with mild cognitive impairment (MCI).

Design and methods: Serum samples were collected from AD, VAD and MCI patients admitted to the University Department of Neurology (Zagreb, Croatia) for regular follow-up. All patients underwent standard neuroimaging procedures including brain CT, neurosonological assessment with intima-media thickness (IMT) and breath holding index (BHI) calculations. Cognitive abilities were tested using standard Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA). Concentrations of KLK6, CLU, ADPN and IL-6 were determined in all serum samples.

Results: We have recruited a total of 235 participants, divided in 4 groups: AD ($N = 70$), VAD ($N = 67$), MCI ($N = 48$) and CHP ($N = 50$). Serum concentrations of KLK6 ($P = 0.137$), CLU ($P = 0.178$) and ADPN ($P = 0.268$) did not differ between AD, VAD, MCI and cognitively healthy control group of participants, whereas IL-6 was significantly higher in VAD patients than in AD, MCI and CHP individuals ($P = 0.014$). There was no association between investigated biomarkers and clinical patient parameters.

Conclusions: Serum concentrations of KLK6, CLU and ADPN do not differ between AD, VAD and controls with and without mild cognitive impairment. Higher IL-6 levels in VAD group point to the inflammatory component in the development of vascular dementia. Investigated biomarkers are not associated with neuroimaging findings and neuropsychological patient data.

1. Introduction

Dementia is a common name for a group of progressive degenerative syndromes which substantially affect the brain and alter memory, behavior, thinking and emotion [1]. Early recognition and timely diagnosis of dementia or even mild cognitive decline is extremely important as it enables opening a potentially larger therapeutic window. Alzheimer's disease (AD) is the most frequent cause of dementia. Clinical hallmark of AD is gradual loss of memory and cognitive capacity, resulting in individuals' inability to perform common everyday activities. Pathobiochemical features of AD are formation of amyloid plaques and neurofibrillary tangles in the hippocampus and enthorinal cortex of the brain [2].Vascular dementia (VAD) is the second most common type of dementia, comprising 15 to 20% of all types of dementia [3,4]. VAD is a common consequence of cerebrovascular disease and vascular risk factors, especially hypertension and diabetes. Besides the memory decline, executive functions of the patients with vascular dementia are often impaired, and they also suffer from depression [5]. Since etiology of dementia may substantially

Abbreviations: AD, Alzheimer's disease; VAD, vascular dementia; MCI, mild cognitive impairment; CHP, cognitively healthy participants; CSF, cerebrospinal fluid; Aβ42, amyloid β42; KLK6, human kallikrein 6; CLU, clusterin; ADPN, adiponectin; IL-6, interleukin-6; IMT, intima-media thickness; BHI, breath holding index; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment.

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impact the patient management and outcome, early diagnosis of the type of dementia in its preclinical stage, is of vital importance. Mild cognitive impairment (MCI) is nowadays increasingly recognized as a disorder that often precedes clinically evident dementia [6]. MCI patients are not demented, but they have mild to minimal memory impairment that does not significantly impact daily functioning.

Recent studies have focused on neuroimaging and determination of amyloid-β42 (Aβ42) or tau-protein concentration in cerebrospinal fluid (CSF) as potential biomarkers for differentiating AD, VAD and MCI [7]. Due to invasive procedure of obtaining the CSF specimen, reliable blood biomarkers for early detection and follow-up of different forms of dementia are needed. Several biomarkers involved in the physiology of the brain tissue have been identified as potential candidates for early diagnosis and differentiation of dementia: human kallikrein 6 (KLK6), clusterin (CLU), adiponectin (ADPN) and inflammatory markers such as interleukin-6 (IL-6). The role of these blood biomarkers in the actual pathology is still controversial. KLK6, also known as neurosin, is serine protease involved in deposition of Aβ42 peptide in brain tissue [8]. It has been shown that blood KLK6 concentration is affected by the advanced age and underlying neurologic pathology [9,10]. Clusterin (CLU) is a chaperone protein which is widely expressed in the brain tissue. Studies on its role in pathogenesis of AD showed that CLU can have opposite effects: cytoprotective effect by preventing the aggregation of Aβ42,and exacerbating effect by enhancing the toxicity of Aβ oligomers [11]. Finally, adiponectin (ADPN) is adipocytokine produced by white adipose tissue which prevents development of cardiovascular diseases and type 2 diabetes. Recent studies indicate that ADPN affects insulin sensitivity, NO production and pro- and anti-inflammatory response in neurodegenerative disorders and VAD [12,13].

We hypothesized that, due to their different pathophysiological roles, serum concentrations of KLK6, CLU and ADPN could differ between subtypes of dementia. Former studies were focused on change in concentration of blood biomarkers in patient group with one diagnosis [9] or there was one blood biomarker assessed in wide range of neurological disorders [14]. Our aim was therefore to assess the difference in concentration of KLK6, CLU and ADPN between AD patients, VAD patients and control group consisted of the cognitively healthy individuals and those with MCI.

2. Materials and methods

2.1. Subjects

Serum samples were collected from AD and VAD patients who were admitted to the University Department of Neurology of the Medical School University Hospital Sestre milosrdnice (Zagreb, Croatia) for regular follow-up during the period from November of 2010 until the April of 2014. Diagnosis of AD was established based on clinical criteria and diagnostic guidelines, as described previously [15]. VAD was diagnosed based on recommendations of the Neuroepidemiology Branch of the National Institute of Neurological Disorders and Stroke (NINDS) and Association Internationale pour la Recherche et l'Enseignement en Neurosciences (AIREN) [16]. There were two control groups: a) control individuals with MCI and b) cognitively healthy individuals. Control individuals in both groups were older than 60 years. MCI was diagnosed according to well known Petersen's criteria [17]. Controls were recruited among spouses of AD and VAD patients included in the study and cognitively healthy patients referred to the University Department of Neurology by their general practitioners for the assessment of other neurological disorders.

2.2. Methods

For each study participant demographic (age, gender, body mass index (BMI), years of education completed during life) and vascular risk factor data (hypertension, diabetes mellitus, coronary disease, atrial fibrillation, smoking) were collected. Current smokers and past smokers who have quitted smoking within the last five years were classified as smokers, whereas non-smokers and past smokers who have quitted smoking more than five years ago — were classified as non-smokers. A patient with systolic blood pressure of 140 mm Hg or higher and/or a diastolic blood pressure of 90 mm Hg or higher, and a patient currently treated with antihypertensive drugs were characterized as patient with hypertension [18]. If a patient was previously diagnosed with type I or type II diabetes, if he/she has at least two random glucose readings of >11.1 mmol/L or fasting blood glucose readings of >7.0 mmol/L, or he/she currently uses standard oral blood sugar lowering drugs or insulin, the patient is considered to have diabetes mellitus [19].

In all study participants, except in controls, brain multi-slice computed tomography (MSCT) was performed (Sensation Multislice Computed Tomography scanner with 16-row detector layer, Siemens, Germany) as a part of the routine diagnostic procedures. Brain MSCT findings were interpreted by neuroradiologist who was blinded to other patient data. Colour Doppler Flow Imaging (CDFI) of carotid arteries and measurement of intima-media thickness (IMT) and beta stiffness index (BSI) was performed on all study participants using the Aloka ProSound-5500 SSD PHD (Aloka Co.Ltd., Tokyo, Japan) with linear 10 MHz transducer according to well defined procedure and diagnostic protocol [20].

Additionally, transcranial Doppler sonography (using TCD Viasys Healthcare with 2 MHz probe, Madison, Wisconsin, USA) was performed in order to assess breath holding index (BHI) as a marker of cerebrovascular vasoreactivity. Increase of blood CO_2, provoked by holding of breath, results in vazodilatory reaction. Extent of reaction is measured by the BHI [21].

In all participants, Mini Mental State Exam (MMSE) and standardized Croatian version of Montreal Cognitive Assessment (MoCA) were used for cognitive assessment [22,23,24]. MMSE is usually used for evaluation of cognitive ability in population of patients with diagnosed dementia. Maximal score is 30 points, indicating absence of dementia, while score lower than 10 indicates severe dementia. MoCA is a test with high sensitivity for MCI with maximal score of 30 points and cut-off score of 26 points indicating MCI. Both tests can conveniently be used in clinical settings. Neurosonological measurements and cognitive testing were performed by experienced neurologists trained in vascular ultrasound and neuropsychological assessment.

Serum blood sample was collected from each study participant on admission. Regular biochemical tests included glucose, uric acid, cholesterol, HDL-cholesterol, LDL-cholesterol and CRP. Analysis was performed on the Beckman Coulter 2700 biochemistry analyzer (Beckman Coulter Inc., Brea, CA, USA) with original Beckman Coulter reagents (Beckman Coulter Biomedical Limited, Co Clare, Ireland). After routine analysis, leftover serum aliquots were immediately stored at −20 °C for KLK6, CLU, ADPN and IL-6 determination. Samples were handled with great care. Analysis was done after thawing. All samples were thawed only once.

Concentration of IL-6 was determined using the IL-6 reagent (Roche Diagnostics GmbH, Mannheim, Germany) on electrochemiluminescence immunochemistry analyzer Cobas e411 (Roche Diagnostics GmbH, Mannheim, Germany). Quality control for IL-6 was done with Preci Control Multimarker 1 and 2 (Roche Diagnostics GmbH, Mannheim, Germany). Data for imprecision (CV) of IL-6 assay are 1.4% at 38.3 pg/mL and 1.4% at 229 pg/mL concentrations.

Adiponectin testing was performed using the immunoturbidimetric assay ADPN (Adiponectin, Randox, Crumlin, United Kingdom) on biochemistry analyzer Beckman Coulter AU 680 (Beckman Coulter Inc., Brea, CA, USA). Quality control was done using Adiponectin Control — Level 2 (ADPN Control 2) (Randox Laboratories Limited, Crumlin, United Kingdom) and Adiponectin Control — Level 3 (ADPN Control 3) (Randox Laboratories Limited, Crumlin, United Kingdom). Within-laboratory CVs for ADPN assay were 2.0% at 4.0 μg/mL and 3.4% at 9.6 μg/mL concentrations.