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Differences in brain cholesterol metabolism and insulin in two subgroups of patients with different CSF biomarkers but similar white matter lesions suggest different pathogenic mechanisms

A. Besga[a,g,*], A. Cedazo-Minguez[b], I. Kåreholt[c], A. Solomon[c,d], I. Björkhem[e], B. Winblad[b], V. Leoni[f], B. Hooshmand[c], G. Spulber[b], A. Gonzalez-Pinto[a,g], M. Kivipelto[c,d], L.O. Wahlund[b]

[a] Department of Psychiatry, Hospital Universitario de Álava, Vitoria, Spain
[b] Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden
[c] Aging Research Center, Karolinska Institutet/Stockholm University, Stockholm, Sweden
[d] Department of Neurology, University of Kuopio, Finland
[e] Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska Institutet, Sweden
[f] Laboratory of Clinical Pathology and Medical Genetics, National Institute of Neurology 'C. Besta', Milano, Italy
[g] Centre for Biomedical Research Network on Mental Health (CIBERSAM), Spain

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ABSTRACT

Investigate possible associations of white matter hyperintensities (WMHs) with the metabolism of cholesterol and insulin in two subgroups of patients with memory complaints and different CSF Aβ42 and CSF tau levels. 59 patients from the memory clinic at Karolinska Hospital were included. Degree of WMHs was rated using the ARWMC scale and the following biomarkers were measured in CSF and plasma: insulin, cholesterol, lanosterol, lathosterol, and oxidized cholesterol metabolites. The WMHs in CSF control-like group correlated with increased brain cholesterol synthesis and reduced efflux of oxysterols and insulin in CSF. In the CSF AD-like group, the WMHs correlated with increased peripheral cholesterol metabolism. Despite having similar appearance on FLAIR images, the pathogenic mechanisms of WMHS are likely to be different in the two groups investigated.

Background

The production and maintenance of myelin are essential for the correct functioning of the brain. Magnetic resonance imaging (MRI) is highly sensitive to lesions affecting the white matter since pathology prolong T2 relaxation by increasing the tissue water content and degrading the myelin. The prolonged T2 relaxation results as areas with high signal hyperintensities on T2-weighted MRI (WMHs).

WMHs represent a gamut of physiologic and pathologic entities observed in a significant proportion of elderly patients. Such changes are present not only in demyelinating diseases, stroke, dementia, but also in normal subjects.

Several models explaining the pathogenesis have been proposed and several mechanisms probably contribute in different group of patients or even within the same patient. In correlative radiologic–pathologic studies, it has been demonstrated that WMHs, though having a uniform appearance on MRI, are histologically heterogenous [4].

Some investigators relate the myelin breakdown to ischemia while others consider it a primary disease process that may be accelerated by or caused by factors such as oligomeric Aβ [10]. There have been many efforts to understand the relationship between amyloid and microglia but still little is known.

Cholesterol and insulin have been suggested to be involved in AD pathogenesis. However, there is evidence that AD pathology affects cholesterol and insulin metabolism [10]. Interestingly, it has been observed that insulin deficiency may impair the ability of oligodendrocytes to form myelin and alterations in oligodendrocytes could also alter their cholesterol production which has clinical implications in AD [6].

The present study aimed to increase our understanding of the role of WMHS in patients with memory complaints by investigating their pathogenesis. We divided a population of elderly patients with memory complaints in two subgroups on the basis of CSF Aβ42 and CSF T-tau levels. The two groups were found to have similar

* Corresponding author at: Department of Psychiatry, Hospital Universitario de Álava, Vitoria, Spain. Tel.: +34 945 00 77 64; fax: +34 945 00 77 64.
E-mail address: ariadna.besgabasterra@osakidetza.net (A. Besga).

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WMHs. In spite of this, significant differences were observed with respect to brain cholesterol metabolism and insulin.

Materials and methods

Subjects

Patients included in the present study were referred to the memory clinic at Karolinska Hospital in Huddinge, Stockholm from primary care centers for the investigation of memory complaints. These patients were all living independently in the community. All subjects underwent a standard protocol including: clinical evaluation, a cognitive and a neuropsychological evaluation, analyses of blood and cerebrospinal fluid (CSF), and brain imaging (MRI). A total of 59 subjects who had available plasma and CSF samples and MRI images were eligible for the study. Subjects with psychiatric disorders (i.e. major depression) or other conditions (i.e. brain tumors) were not considered for this study. There were no significant differences in age and sex between the excluded and the included patient groups. For the aim of the study, patients were classified according to CSF Aβ42 and CSF T-tau levels.

MRI scans and WMHs rating

MRI scans were performed at Karolinska Institutet, Stockholm. A 1.5 T scanner was used. A series of T2 weighted images, axial fluid-attenuated inversion recovery (FLAIR) images and coronal and sagittal 3D T1 sequences were acquired. All the scans were collected centrally and the rating of WMHs was performed by one independent rater, who was blinded to all clinical data. All ratings were performed on FLAIR images, applying a semi-quantitative method of Wahlund et al. [15]. Five regions were rated in the left and right hemispheres separately: frontal, parietooccipital, temporal and infratentorial and basal ganglia. Except for basal ganglia the rating was done on a scale ranging from 0 to 3 (0:no lesions, 1:focal lesions, 2:increasing confluence and 3:diffuse involvement). Changes on the basal ganglia were rated in a similar way (0: no lesions; 1: focal lesions ($\geq$5 mm); 2: more than one focal lesion and 3: confluent lesions). Total WMHs for the whole brain was calculated by summing the ratings from all the brain regions from both hemispheres. The total WMHs score was used to address participants belonging to four different groups as a function of their severity: no WMHS (0 points), mild WMHs (1 point), moderate WMHs (2 points) and severe WMHs ($\geq$3 points).

Biochemical analyses

CSF was collected for diagnostic purpose by lumbar puncture in polypropylene tubes, gently mixed to avoid gradient effects and centrifuged at $2000 \times g$ for 10 min. Aliquots were stored at $-80\,^{\circ}$C until the biochemical analysis. Tau was determined using a sandwich enzyme-linked inmunoabsorbent assay (ELISA) and P-tau (P-Thr 181) was determined using a sandwich ELISA, with monoclonal antibody HT7 (recognizing all forms of Tau) used as capturing antibody, and AT270 (specific to PThr 181 P-tau) used as a detection antibody [14]. Aβ42 was determined using a sandwich ELISA specific for Aβ42 as previously described [1]. All kits were purchased from Innogenetics NV, Ghent, Belgium. APOE levels in CSF were assayed by a slight modification of a commercial immunoassay method for APOE.

CSF levels of cholesterol, 24S-hydroxycholesterol (24S-OH) and 27-hydroxycholesterol (27-OH) were assayed by isotope dilution-mass spectrometry. In plasma sample collected contextually also 24S-OH, 27-OH, lanosterol and lathosterol were assayed by isotope dilution-mass spectrometry [9] and total, LDL- and HDL-cholesterol were measured using a standard enzymatic assay (Modular Analytics; Roche Diagnostics, Germany). Insulin levels were measured by a standard radioimmunoassay with high sensitivity (Millipore MA, USA).

Statistical method

Ordered logistic regressions were used to analyze the severity of WMHs. The results are presented as β-coefficients and *p*-values.

A combination of low CSF Aβ42 and high CSF T-tau levels predicts the presence of AD pathologic with high accuracy [12]. Considering this we have categorized the patients according to a combination of CSF Aβ42 and CSF T-tau. The variable combining low CSF Aβ42 and high CSF T-tau has been created by standardizing both variables and reverting Aβ42 by multiplying it with minus one, and then adding the two variables. Interaction terms between this combined variable and variables related to insulin and cholesterol metabolism have been included in the analyses presented in Table 4. The interaction terms have been modeled in such ways that the results show the results for the 90th percentile (indicated as high CSF Aβ42 and low CSF T-tau in Table 4) and 10th percentile (indicated as low-high) of this variable.

Ethical aspects

The local Ethics committee at the Huddinge University Hospital approved the study. All the patients (or tutors) gave their informed consent to participate in the study, which was conducted according to the provisions of the Helsinki Declaration.

Results

The population is divided according to the median of the combination of (low) CSF Aβ42 and (high) CSF T-tau.

Table 1 describes the characteristics of the patients. The CSF AD-like group was older with lower results in cognitive tests and with higher levels of global deterioration measured with the Global Deterioration Scale (GDS). Other socio-demographic or clinical characteristics did not differ significantly between the groups. Interestingly there were no significant differences in vascular risk factors.

WMH burdens were greater in CSF AD-like group, but this difference did not reach statistical significance (Table 2).

The biochemical characteristics of the study population are presented in Table 3. There were significant differences between both groups in R_lanosterol, lanosterol, 24S-OH and 27-OH.

Table 4 shows the relationships between WMHs and the markers for metabolism of cholesterol and insulin in both study groups.

The associations between WMHs and levels of CSF Aβ42, T-tau and P-tau in each group were also analyzed with ordered logistic regressions as in Table 4, for control-like group significant associations were found between T-tau ($p = 0.015$), F-tau ($p < 0.0001$) and WMHs in the basal ganglia, and a borderline significant association between Aβ42 ($p = 0.068$) and WMHs in the basal ganglia. For AD-like group, only the association between Aβ42 ($p = 0.034$) and WMHs for the frontal region was significant.

Discussion

This study combined quantitative metabolic analysis and brain MRIs to investigate the pathogenesis of WMHs in elderly patients with memory complaints.

The population was divided according to the median of the combination of CSF Aβ42 and T-tau levels, used as biomarkers to identify patients with probable AD from controls in a memory clinic