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Platelet Tau Pattern Correlates with Cognitive Status in Alzheimer's Disease

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Abstract. Platelets are major reservoirs of circulating amyloid-β and amyloid-β protein precursor (AβPP) and have been postulated as a reliable source for biological markers of Alzheimer's disease (AD). We have recently demonstrated that tau is also present in platelets, and that there are differences in the electrophoretic patterns of platelet tau forms in AD subjects with respect to controls. Here, we demonstrate that modifications in platelet tau forms occur independently of age in a broad population of 104 neurologically healthy individuals. More interesting, a strong correlation of platelet markers with the degree of cognitive impairment was evidenced in a group of 47 AD patients in comparison with 19 cognitive healthy subjects. In our series, platelet tau forms ratio had a sensitivity of 75.7% and specificity of 73.7%, respectively. We also found that platelet tau displays a significantly higher correlation with the presence of AD than the analyses of platelet AβPP.

Keywords: Alzheimer's disease, cognitive impairment, human platelets, tau biomarker

INTRODUCTION

Biomarkers belong to an impending field in AD research since they may support clinical and neuropsychological diagnosis and even allow preclinical diagnosis [1]. The last AD criteria [2] consider two kinds of biomarkers, those related to Aβ accumulation and those related to neuronal injury, including those that are based on tau protein measures. In any case, the search for reliable, non-invasive, and non-expensive biomarkers has been an important drive for biomarker research. Platelets have been postulated as a peripheral marker for AD since they carry 95% of circulating amyloid-β protein precursor (AβPP). The ratio of 130 and 110 kDa forms of AβPP is modified in AD and has been postulated to correlate with the presence of AD, so evaluations of AβPP have been suggested as a reliable non-invasive disease biomarker [3], as well as a sensor for treatment response [4, 5]. We have recently found that tau protein is also present in platelets, and there are different molecular weight forms of tau that are differentially expressed as evidenced from immunoblots of blood samples of AD patients and healthy controls [6]. In fact, we described that high molecular weight forms of tau—that appear to be aggregated forms of this protein—are found in a significantly high proportion in platelets from AD subjects, so the ratio of high molecular weight forms of tau (>80 kDa) to low molecular weight monomeric forms of the protein (HMWtau/LMWtau) is significantly higher in AD patients.

To evaluate whether platelet tau ratio may serve as a novel biomarker for AD, we evaluated if platelet tau patterns may be affected by age in a group of control subjects, then we determined if platelet tau modifications correlate with AD presence and/or cognitive

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status in AD and control subjects. Finally we also analyzed the AβPP fractions in platelets of the same AD and control subjects and searched for a relation between platelets tau and AβPP pattern.

METHODS

Subjects

In the first stage, 104 cognitively healthy subjects (Mini-Mental Status Exam (MMSE) score >25) of ages ranging from 29 to 87 years old were recruited at Peñaflor Hospital, Chile, for analyses of platelet tau at different ages. In the second stage, 47 probable AD subjects [7] and 19 cognitive healthy subjects were recruited from the Cognitive Neurology & Dementia Unit (Unidad de Neurología Cognitiva y Demencias) of the Neurology Service, Hospital del Salvador, Santiago, Chile. Subjects were evaluated with MMSE [8] and global deterioration scale [9]. In addition, all of the AD subjects were evaluated with a brain computed axial tomography or magnetic resonance and basic biochemical laboratory tests to discard conditions other than AD that may affect cognitive performance.

The procedures were approved by the Committee on Ethical Issues of Hospital Salvador (Santiago, Chile) and the Medical Ethics Committee of the ICC, and all of the participants and/or their legal guardian signed informed consents.

Platelet tau analyses

5 ml of peripheral blood were obtained from each subject and analyses were carried out as previously described [6]. Briefly, platelet rich plasma was obtained by centrifugation at $200 \times g$ for 10 min at room temperature in a Rotina 35R Hettich zentrifugen. Platelets were then isolated by centrifugation at $1600 \times g$ for 10 min, resuspended in NH_4Cl 0.83% at room temperature for 5 min, and then centrifuged at $1500 \times g$ for 10 min. Platelet pellets were washed twice in phosphate buffered saline (1.4 mM NaCl, 0.02 mM KCl, 0.1 mM Na_2HPO_4, 0.017 mM KH_2PO_4) −1 mM EDTA. Platelets were lysed in 150 μl RIPA lysis buffer (5.0 mM Tris-HCl pH 7.5, 1.5 mM NaCl, 10% NP-40, 10% deoxycholate, 20 mM EDTA pH 8.0, 500 mM NaF, 1% SDS) and 2 μl of a protease inhibitor cocktail (Sigma). A final centrifugation step at $1500 \times g$ for 10 min at 4°C was carried out and the supernatant protein concentration was determined by Bradford method (BioRad) according to the manufacturer's instructions.

50 μg of total platelet protein were loaded per well in 10% acrylamide gels and electrophoresis was performed for 90 min at 100 mV. Proteins were then transferred to nitrocellulose membranes for 90 min at 80 mV by tank transfer. Membranes were blocked with 5% non fat dry milk for 1 h at 25°C and probed with the following primary antibodies: 2 μg/ml Tau-5, 2 μg/ml Tau-1 (both generous gift from Dr. Lester Binder, Northwestern University, Chicago, IL), PHF-1 1 : 100 (generous gift from Dr. Peter Davies, Albert Einstein College of Medicine, NY, USA), and 22C11 0.25 μg/ml (Chemicon). Tau-5 recognizes every form of tau phosphorylated and non-phosphorylated, while Tau-1 reacts with tau when non-phosphorylated at Ser 195/198/199/202 sites. PHF-1 recognizes tau phosphorylated at Ser 396 and Ser 404. On the other hand, 22C11 antibody reacts with 66 to 81 N-terminal residues from every isoform of AβPP. Immunoreactive bands were detected by using Super Signal West Pico chemioluminescent substrate (Pierce, Rockford, IL) in Fuji X-ray films. Films were scanned and band intensities quantified using the Image J 1.40 software (National Institutes of Health, USA).

Statistical analysis

Means were compared with unpaired samples *t* test or one-way ANOVA with Bonferroni *post hoc* test for different groups. Multivariate analyses were performed by forward stepwise binary logistic regression. All the analyses were performed with SPSS 17.0 software for Windows. Significance level was set on 0.05.

RESULTS

Platelet tau was obtained from blood samples of different ages individuals without memory complains. Subjects' ages ranged from 29 to 87 years (mean 57.7 ± 12.2 years). Immunoblots for platelet tau analyses were performed with Tau-1, Tau-5, and PHF-1 antibodies. No correlation between platelet tau ratio and age was found when analyses were performed with either Tau-1 or Tau-5 for non-phosphorylated forms and total forms of tau, respectively (Fig. 1A). When probed with PHF-1 antibodies, we also found no differences in the HMWtau/LMWtau ratio (not shown). Interestingly, when analyzing total quantities of phosphorylated tau relative to β-actin we found that phosphorylated tau quantities tend to decrease with age (Fig. 1B).

In another study, we investigated platelet tau variants as related to the cognitive status of a population

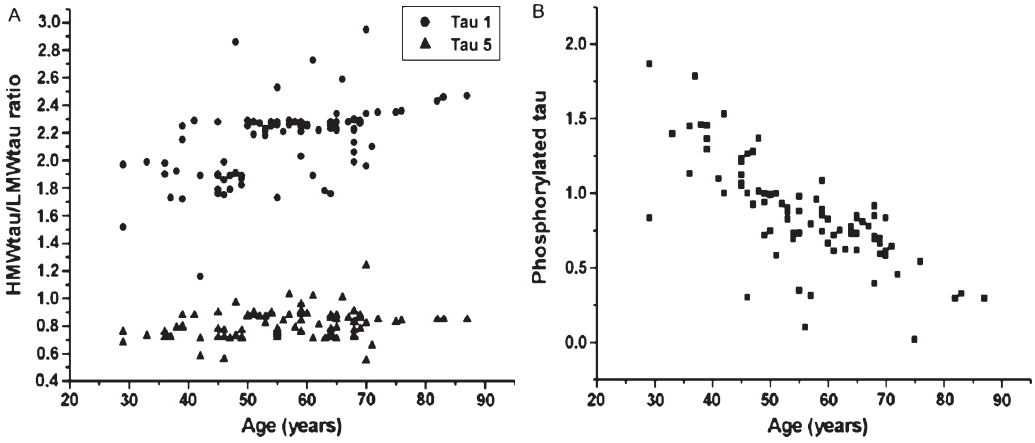

Fig. 1. Evaluation of platelet tau at different ages. A) HMWtau/LMWtau ratio show no significant variation with age in control subjects. Black triangles correspond to immunoblots with Tau-5 antibody ($R^2 = 0.3089$). Circles correspond to the same samples analyzed with Tau-1 antibody ($R^2 = 0.0235$). B) Phosphorylated tau levels in platelets decrease with age. Squares correspond to phosphorylated tau levels measured with PHF-1 antibody and normalized with β-actin.

Table 1
Demographic and clinical features of the groups of AD and control subjects. $^*p < 0.05$

	Control	AD	p
Male (number of subjects)	5 (19%)	21 (78%)	0.167
Female	14 (31%)	26 (58%)	0.167
Age	70 (52–82)	76 (61–89)	0.014*
Education (years)	11 (4–17)	8 (1–17)	0.041*
Global deterioration scale	1 (1–2)	5 (4–6)	0.000*
MMSE	29 (22–30)	16 (4–26)	0.000*

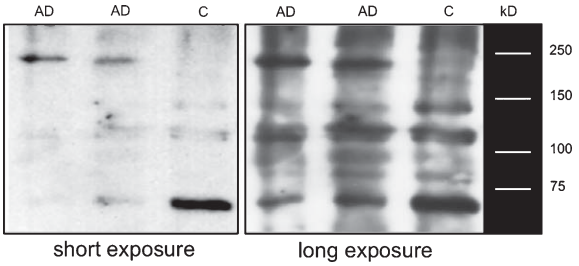

Fig. 2. Representative immunoblots of platelet tau with Tau-5 antibody. In AD subjects (AD), high molecular weight forms of tau appear early on short exposure times, while in control subjects (C) tau monomers are more noticeable (left image, lower molecular weight band). At long exposure times (right image) high and low molecular weight forms of tau can be appreciated in control and AD samples, but platelets HMWtau/LMWtau ratio is consistently higher in AD subjects.

of patients and control healthy subjects incorporated into this study. The demographic and cognitive characteristics of AD and control subjects are shown in Table 1.

Platelet tau ratio was obtained for controls and AD subjects as described by Neumann et al. [6]. Figure 2 shows that HMWtau appears in shorter exposure times and are more conspicuous in relation to LMWtau in AD subjects. Densitometric analyses of immunoblots show that HMWtau/LMWtau ratio is significantly higher in the AD group in relation to control subjects ($p = 0.001$) (Fig. 3A). No major statistical differences were found in tau ratios at different stages of the disease, but there is a notorious tendency to higher HMWtau/LMWtau ratios as AD progresses (Fig. 3B).

Since there are some differences between control and AD groups (as seen in Table 1), we decided to evaluate which variables were predictors of cognitive state (AD or control) in a model generated by binary logistic regression. This model incorporates only two explanatory variables: HMWtau/LMWtau ratio ($p = 0.047$) and years of education ($p = 0.048$), while other variables like age and gender where not incorporated. Figure 4 shows the Receiver-Operating Characteristic curve for platelet tau ratio as a biomarker for AD. In this analysis, a cut-off point of 1.11 for HMW-tau/L-tau ratio displayed a sensitivity of 75.7% and a specificity of 73.7% to discriminate AD and control subjects.

On the other hand, when immunoblots with 22C11 antibody where carried on in the same samples and evaluation of AβPP form ratio was performed as previously described [4, 10], we found no significant correlation between platelet AβPP form modification and platelet tau ratio or subject's cognitive state (not shown).

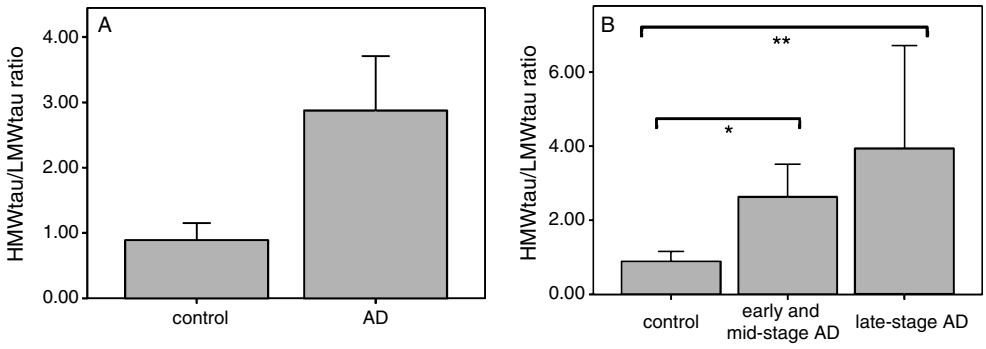

Fig. 3. A) Platelets HMWtau/LMWtau ratio is elevated in AD samples when compared to control samples ($p = 0.001$). B) This difference can also be appreciated when AD subjects are grouped in mild to moderate disease ($^{*}p = 0.016$) and advanced AD ($^{**}p = 0.004$). Bars represent 95% confidence intervals.

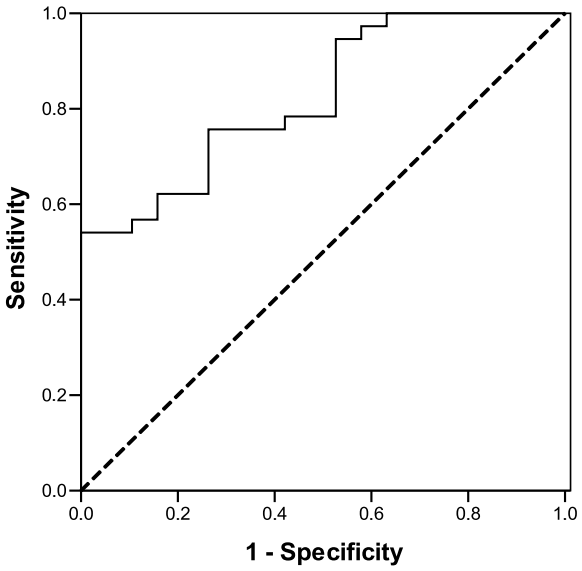

Fig. 4. Receiver-Operating Characteristic curve showing sensitivity and 1-specificity of platelets tau ratio as an AD biomarker. Area under the curve corresponds to 0.824 (IC 95: 0.716–0.932).

DISCUSSION

The need for AD biomarkers has been raised for several years and the requisites for a useful biomarker have been postulated [11]. Unfortunately, principal requirements for such a biochemical marker—to be reliable, non-expensive, and non-invasive—have not been met. There is interesting evidence pointing to peripheral cells as possible sources for new biomarkers, since many modifications can be found in those cells in AD [12–14]. Although AβPP has been proposed as a reliable AD biomarker, there are some requirements in sample management that may affect the results, including time for sample processing, and this may be the motive for our negative results with this marker. On the other hand, we confirmed our previous data that platelet tau suffer modifications in AD. In fact, we show that platelet tau modifications are age independent and may serve as an adequate biomarker that may even serve to monitor disease progression since alterations in platelet tau ratio are more significant in advanced stages of the disease. This relationship between cognitive impairment and alterations in platelet tau may be important to monitor advances of pathological processes in AD and also may be useful for follow up responses to current and future therapies. On the other hand, there is still the need to evaluate platelet tau modifications at very early or preclinical stages of AD. This will have to be evaluated in future trials with large cohorts of elderly subjects or in patients at early stages of AD.

The reason for platelet tau modifications within AD is still not clear, but it can be postulated that the mechanism by which tau ratio suffer modifications in AD platelets may be related to posttranslational modifications and tau oligomerization in a similar way as occurs in the central nervous system of AD patients [15]. On the other hand, phosphatase-kinase balance may be affected by aging and/or oxidative stress [16] and may account for the observed decrease in phosphorylated platelet tau in relation to age.

Since many changes in immunomodulation [17, 18] and metabolism [19, 20] have been related to AD, it is not unlikely that these same insults may act at a systemic level producing modification in peripheral cells that are similar to the process occurring at neurons and glial cells in central nervous system.

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