Inability of Plasma and Urine F2A-Isoprostane Levels to Differentiate Mild Cognitive Impairment from Alzheimer's Disease

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Key Words

Alzheimer's disease biomarkers · Isoprostane · Mild cognitive impairment · Oxidative stress

Abstract

Background: Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease (AD). The pathobiological changes related to AD occur long before the overt clinical symptoms. The plasma lipid peroxidation enzyme F2-isoprostane has been suggested as a biomarker to detect the progression from mild cognitive impairment (MCI) to AD. **Objective:** To test whether plasma and urine F2-isoprostane was diagnostic for dementia in living people. **Methods:** Plasma and urine were collected from 222 Religious Orders Study participants with a clinical diagnosis of no cognitive impairment, MCI or AD at time of fluid collection. Isoprostane levels were determined using gas chromatography/mass spectroscopy. **Results:** Plasma and urine F2-isoprostane levels did not differ between the three clinical groups. Postmortem neuropathologic diagnosis of subjects who died during the course of the study was not associated with baseline blood or plasma F2-isoprostane levels. **Conclusions:** In living people, plasma or urine isoprostane levels were not sensitive enough to discriminate between individuals with a clinical diagnosis of no cognitive impairment, MCI or AD.

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Introduction

Increases in brain markers of oxidation and reduced activities of antioxidant enzymes implicate oxidative damage in the pathobiology of Alzheimer's disease (AD). Isoprostanes which arise from free radical-mediated peroxidation of polyunsaturated fatty acids were elevated in brain tissue and cerebral spinal fluid (CSF) obtained at autopsy in advanced and early AD [1–7], as well as in CSF harvested intra vitam from sporadic and familial AD [6, 8]. Since obtaining CSF via a spinal tap can be stressful and not easily obtained in most clinics, investigators have quantified isoprostanes in plasma and urine with studies showing increased [6] or no change [6, 7] in early and late stage AD. To assist in resolving this issue, we measured plasma and urine F2-isoprostane levels from a cohort of living people with a clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) or AD.

Subjects and Methods

Subjects

In 2003, plasma and urine samples were obtained antemortem from participants in the Religious Orders Study, a longitudinal clinical-pathological study of aging and AD [1, 9]. Clinical diagnoses of AD dementia, MCI and NCI at the time of sample collection are based upon evaluation closest to fluid collection using previously described criteria [9]. At baseline, 167 (75%) of the subjects were clinically classified as NCI, 34 (15%) as MCI, and 21

Table 1. Clinical characteristics of living subjects

Characteristic	Baseline clinical diagnosis				Comparison by diagnosis group, p
	NCI (n = 167)	MCI (n = 34)	AD (n = 21)	total (n = 222)	
Males	44 (26%)	8 (24%)	7 (33%)	59 (27%)	0.720 ^a
Years of education ^b	17.7 ± 3.0	17.6 ± 3.1	18.3 ± 3.4	17.8 ± 3.0	0.141 ^c
ApoE ε4 alleles	39 (24%)	12 (35%)	10 (48%)	61 (28%)	0.044 ^a
Age at blood draw, years ^b	79.8 ± 6.3	83.5 ± 5.9	86.3 ± 7.0	80.9 ± 6.6	<0.0001 ^c
MMSE at blood draw ^b	29.0 ± 1.3	27.4 ± 2.2	16.1 ± 7.7	27.6 ± 4.6	<0.0001 ^c
Global cognitive score ^b	0.4 ± 0.5	-0.3 ± 0.5	-2.0 ± 0.9	0.1 ± 0.9	<0.0001 ^c
Subjects on statins	50 (30%)	10 (29%)	3 (14%)	63 (28%)	0.32 ^a
Urine isoprostane, ng/mg creatinine ^{d,e}	2.8 ± 126%	2.6 ± 170%	3.9 ± 105%	2.8 ± 132%	0.246 ^c
Plasma isoprostane, pg/ml ^{d,e}	107 ± 73%	111 ± 87%	138 ± 70%	109 ± 75%	0.250 ^c

MMSE = Mini-Mental State Exam.

^a χ^2 test; ^b Mean ± SD; ^c Kruskal-Wallis test; ^d ANOVA; ^e GM ± GCV.

(9%) as AD. Subjects who died during the course of the study received a consensus conference final clinical diagnosis and their brains were neuropathologically examined according to CERAD [10], NIA-Reagan criteria [11] and Braak scoring [12]. ApoE genotyping was performed as previously reported [13].

Isoprostane Levels

Plasma and urine were cooled to 4°C, spun within 15 min, aliquoted as 960 µl plasma with 40 µl 20× protease inhibitor solution (Complete; Roche, Indianapolis, Ind., USA), and stored at -80°C until measurement [2–5, 13]. Levels of isoprostane iPα-VI (F2A) were determined by gas chromatography/mass spectroscopy [2–5, 13]. The inter- and intra-assay variability of the assays was approximately 5%.

Statistical Analysis

Since F2-isoprostane levels displayed a skewed distribution, the data are presented as the geometric means (GM) and geometric coefficients of variation (GCV), which are presented as GM ± (GCV; percentage difference from the mean) [14]. F2-isoprostane, neuropathologic and demographic data were compared using analysis of variance (ANOVA) or Kruskal-Wallis test, gender distribution by a χ^2 test, and association of plasma with urine F2-isoprostane levels by the Spearman's rank correlation coefficient. Ordinal logistic regression examined the relationship between F2-isoprostane and neuropathologic data. Statistical significance was set at $\alpha = 0.05$.

Results

At baseline, the mean age of the NCI (79.6 years) subjects was significantly less than that of both MCI (83.5 years) and AD (86.3 years) subjects (Kruskal-Wallis, $p < 0.0001$). Mini-Mental State Exam scores differed significantly

among the groups (Kruskal-Wallis, $p < 0.001$); post-hoc testing showed that the means for the NCI (28.9) and MCI (27.4) groups did not differ significantly from each other, but both were significantly higher than that of the AD (16.2) group (table 1). ApoE ε4 allele status differed significantly between the three clinical groups ($\chi^2 = 6.25$; $p = 0.044$; table 1). Plasma F2-isoprostane levels were not associated with age, education, sex or use of statins ($p > 0.15$); urine levels were higher for males ($p = 0.026$, Spearman correlation) and those on statins had lower urine isoprostane levels ($p = 0.0244$). F2-isoprostane levels were determined for 134 (74%) NCI, 32 (18%) MCI and 14 (8%) AD cases. Plasma F2-isoprostane was not measured in 33 (22%) NCI, 2 (6%) MCI, and 7 (33%) AD subjects. The overall GM ± GCV for plasma F2-isoprostane was 107 pg/ml ± 73%, and 2.80 ng/mg ± 132% for urine creatinine. Plasma and urine F2-isoprostane levels were not correlated (Spearman correlation, 0.67, $p < 0.0001$) and did not differ among the three clinical groups at the time of specimen draw. Mean urine levels were 2.8 ng/mg creatinine for NCI, 2.6 ng/mg creatinine for MCI and 3.7 ng/mg creatinine for AD, and plasma F2-isoprostane levels were 107 pg/ml for NCI, 111 pg/ml for MCI and 138 pg/ml for AD.

A consensus postmortem clinical evaluation of the 63 deceased subjects revealed 22 as NCI (2 were MCI at baseline; 20 were NCI), 15 as MCI (6 were NCI at baseline; 9 were MCI) and 26 as AD (14 were AD at baseline, 5 were MCI and 7 were NCI; table 2). Although these clinical groups did not differ in Braak staging, by NIA-Reagan diagnosis the AD group was neuropathologically more

Table 2. Neuropathological characteristics of deceased subjects

Neuropathology	Consensus postmortem clinical diagnosis				Comparison by diagnosis group	Pairwise comparisons
	NCI (n = 22)	MCI (n = 15)	dementia (n = 26)	total (n = 63)		
Distribution of Braak scores						
0	1	0	0	1	p = 0.291 ^a	–
I/II	6	3	2	11		
III/IV	14	9	17	40		
V	1	3	7	11		
Distribution of NIA Reagan diagnosis (likelihood of AD)						
No AD	0	0	0	0	p = 0.004 ^a	NCI < AD
Low	13	9	6	28		
Intermediate	9	4	15	28		
High	0	2	5	7		
Distribution of Modified CERAD diagnosis						
No	7	7	3	17	p = 0.008 ^a	(NCI, MCI) < AD
Possible	3	2	3	8		
Probable	11	4	10	25		
Definite	1	2	10	13		

^a Kruskal-Wallis test.

advanced compared to NCI (ANOVA, $p = 0.004$), whereas using CERAD criteria the AD group was more advanced neuropathologically than both NCI and MCI subjects (ANOVA, $p = 0.0008$; table 2). Postmortem neuropathology was not associated with plasma F2-isoprostane levels based upon ordinal logistic regression models.

Discussion

Despite differences in ages between the NCI, MCI and AD subjects examined at time of specimen collection, we found no difference in plasma and urine F2A-isoprostane levels, consistent with other reports [6, 15]. By contrast, others report elevated brain, CSF and plasma F2-isoprostane levels in MCI and AD [6], suggesting that F2-isoprostane levels could act as a biomarker for AD. The inconsistency in using peripheral compared to CSF F2-isoprostane level as a biomarker for AD remains to be determined. Since every cell produces F2-isoprostane, peripheral production unrelated to a particular neurodegenerative disease may easily confound the usefulness of plasma and urine F2-isoprostane levels as a biomarker for AD. Moreover, F2-isoprostane quantification is sensitive to various confounding variables including diet, exercise, body mass index and other diseases associated with lipid peroxidation [16]. These factors as well as methods of

measurement vary across studies and could possibly underlie the inconsistency in peripheral F2-isoprostane measurements. However, a comparative study using a variety of analytic measures of plasma and urine F2-isoprostane failed to reproducibly demonstrate an increase in isoprostane levels in AD versus controls [6].

Although we found that postmortem neuropathologic diagnosis of subjects who died since entering the study was not associated with baseline plasma F2-isoprostane levels, brain F2-isoprostane levels correlate with reduction in brain weight and Braak staging [13, 17]. These findings suggest that brain, but not peripheral F2-isoprostane levels more accurately reflect the burden of underlying AD pathology rather than clinical status. Despite these neuropathological observations, as first reported by Montine et al. [18], plasma F2-isoprostane levels do not appear to be a sensitive biomarker for the clinical progression of AD.

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