Peripheral Oxidative Stress Biomarkers in Mild Cognitive Impairment and Alzheimer’s Disease

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Accepted 18 March 2011

Abstract. Oxidative stress has been associated with normal aging and Alzheimer’s disease (AD). However, little is known about oxidative stress in mild cognitive impairment (MCI) patients who present a high risk for developing AD. The aim of this study was to investigate plasma production of the lipid peroxidation marker, malonaldehyde (MDA) and to determine, in erythrocytes, the enzymatic antioxidant activity of catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) in 33 individuals with MCI, 29 with mild probable AD and 26 healthy aged subjects. GR/GPx activity ratio was calculated to better assess antioxidant defenses. The relationship between oxidative stress and cognitive performance was also evaluated by the Mini Mental State Examination (MMSE). AD patients showed higher MDA levels than both MCI and healthy elderly subjects. MCI subjects also exhibited higher MDA levels compared to controls. Catalase and GPx activity were similar in MCI and healthy individuals but higher in AD. GR activity was lower in MCI and AD patients than in healthy aged subjects. Additionally, GR/GPx ratio was higher in healthy aged subjects, intermediate in MCI and lower in AD patients. No differences in GST activity were detected among the groups. MMSE was negatively associated with MDA levels (r = −0.31, p = 0.028) and positively correlated with GR/GPx ratio in AD patients (r = 0.68, p < 0.001). MDA levels were also negatively correlated to GR/GPx ratio (r = −0.31, p = 0.029) in the AD group. These results suggest that high lipid peroxidation and decreased antioxidant defenses may be present early in cognitive disorders.

Keywords: Aging, Alzheimer’s disease, antioxidant enzymes, malonaldehyde, mild cognitive impairment, oxidative stress

INTRODUCTION

Over the last decade, investigations into the aging process among individuals with cognitive impairment, such as mild cognitive impairment (MCI) and Alzheimer’s disease (AD), have aroused special interest due to the growing burden of neurodegenerative diseases. One of the main theories of Alzheimer’s disease pathogenesis focuses on oxidative stress, which is an imbalance between the production of reactive oxygen species (ROS) and the body’s ability to neutralize them. ROS can react with lipids, proteins, and nucleic acids, leading to structural damage and functional impairment, including cognitive impairment.

This study aimed to investigate plasma production of malonaldehyde (MDA), a marker of lipid peroxidation, and to determine the enzymatic antioxidant activity of catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) in individuals with mild cognitive impairment (MCI), mild probable Alzheimer’s disease (AD), and healthy elderly subjects. The relationship between oxidative stress and cognitive performance was also evaluated by the Mini Mental State Examination (MMSE). The results indicate that high lipid peroxidation and decreased antioxidant defenses may be present early in cognitive disorders.
interest. Although both entities are age-associated disorders, MCI has been conceptualized as a transitional state between normal aging and dementia [1]. In fact, subjects with MCI present high risk for developing AD [1–3] and there is a growing interest in investigating early peripheral biomarkers in a bid to develop preventive strategies and therapeutic actions. In fact, investigation of oxidative stress markers in elderly with cognitive impairment has become a focus in the field of behavior and cognition. According to the “free radical theory of aging”, proposed by Denham Harman in 1956 [4], aging is a result of the accumulation of biomolecules damaged by free radicals, produced during normal metabolism, such as the superoxide anion (O$_2^·$) and hydroxyl radical (OH·). These reactive species can cause oxidative damage to proteins, lipids and nucleic acids, leading for example to lipid peroxidation and DNA mutations [5, 6].

Strong evidence supports the role of oxidative damage in aging [7–10] as well as in MCI and AD [11]. Nunomura et al. [12] showed that oxidative stress precedes the development of the neuropathological hallmarks of AD such as the extracellular senile plaques formed by amyloid-β peptide (Aβ) deposition, and the neurofibrillary tangles consisting of abnormally phosphorylated tau protein. Aβ deposition has also been associated with neuronal lipid peroxidation, protein oxidation and DNA oxidation in animal models of AD [13].

Oxidative stress parameters have been studied in several matrices. In AD patients it was detected in brain tissue, cerebrospinal fluid (CSF), and in blood, showing increases in: 1) protein oxidation markers such as carbonyl proteins [14–16] and 3-nitrotyrosine [17]; 2) DNA and RNA oxidation, evaluated by levels of 8-oxo-2′-deoxyguanosine (8-oxodGuo) and 8-oxo-guanosine (8-oxoGua) [17–21]; 3) lipid peroxidation products, such as malonaldehyde (MDA) [22–26], isoprostanes [27–29], and 4-hydroxy-2-nonenal [30]. Regarding MCI, 8-oxoGua [31], carbonyl proteins and MDA [32] were described in brain tissue; 8-oxodGuo in leukocytes [20]; isoprostanes in CSF, plasma and urine [29] and MDA, and carbonyl protein in serum/plasma [14, 26, 33]. Although evidence suggests oxidative damage in MCI, it is unclear if these alterations are the same as those observed in AD or whether MCI could be a biochemical transition stage between healthy aging and AD.

In addition to the potential role of oxidative stress markers, the investigation of antioxidant enzymes in cognitive impairment are crucial considering their essential role in maintaining oxidants within physiological levels thereby avoiding damage to neuronal cells. The evaluation of antioxidant defenses in MCI and AD can yield valuable information about early changes in the course of the disease. Although several studies [14, 26, 33–35] have evaluated the activity of these enzymes in the two disorders, the findings are controversial. Furthermore, the relationship between oxidative stress and cognitive performance in both MCI and AD subjects is not yet fully understood.

In this context, the aim of the present study was to investigate the presence of lipid peroxidation based on plasma MDA levels and to measure the activity of catalase, glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) antioxidant enzymes in erythrocytes of healthy elderly, MCI, and AD subjects. The study also investigated the relationship between oxidative stress and cognitive performance. Our hypothesis was that there is an imbalance between prooxidant and antioxidant defenses in MCI and AD subjects which varies over the course of cognitive impairment.

**METHODS**

**Subjects**

Twenty-nine patients aged 66–89 years with probable AD according to the NINCDS/ADRDA criteria [36], with mild dementia (Mini-Mental State Examination-MMSE-score 18 to 26) were included. The MCI group comprised 33 individuals aged 61–89 years, 26 of whom had single domain amnestic MCI and 7 with multiple domain amnestic MCI. The following inclusion criteria applied: (a) memory complaint, corroborated by an informant; (b) objective memory impairment (and of other cognitive functions in the multiple domain amnestic MCI cases) detected by neuropsychological evaluation; (c) largely intact general cognitive function; (d) essentially preserved activities of daily living; and (e) non demented [1]. For the neuropsychological evaluation, the Brazilian version of the Mattis Dementia Rating Scale [37, 38], Visual Reproduction and Logical Memory test of the Wechsler Memory Scale were employed [39].

The age-matched control group comprised 26 individuals aged 62 to 83 years with no memory complaints and with 0 score on the Clinical Dementia Rating (CDR = 0) randomly chosen from the general population drawn from participants in socio-cultural activities for the elderly.

In order to assess global cognitive performance, the subjects from the three groups were evaluated by the
MMSE. All individuals included in this study were non-smokers and were in a controlled clinical and nutritional status (19 < BMI < 25) evaluated by clinical examination and routine laboratory test, such as blood count, thyroid functions, and chemistry serum levels. The presence of clinically-controlled comorbidities such as diabetes mellitus, hypothyroidism, hypertension, and dyslipidemia was accepted. All patients were in stable conditions and without acute co-morbidities at the time of sample collection. None were in use of anticholinesterasic drugs or antioxidant supplements.

The clinical part of this study was performed at the Behavioral and Cognitive Neurology Unit, School of Medicine, University of São Paulo. This study was approved by the Institutional Ethics and Research Committee and all subjects or responsible caregivers, as appropriate, signed informed consent forms.

Laboratory determinations

Blood sampling

Blood (20 mL) was drawn from an antecubital vein into heparin anticoagulant tubes and centrifuged at 700–1000 g (10 min/4 °C). Plasma was separated and stored at −80 °C. Erythrocytes were washed three times with saline solution and then hemolysated by the addition of distilled water (1:5) and stored at −80 °C.

Lipid peroxidation evaluation

Lipid peroxidation, the oxidative deterioration of polyunsaturated fatty acids [40], was assessed by plasma MDA production [41, 42]. Plasma MDA was reacted with thiobarbituric acid in an acid medium at 90 °C for 1 h. Afterwards, centrifugation for protein removal, supernatant was filtered through a 0.2 μm membrane and the colored complex was analyzed by reverse-phase high performance liquid chromatography (HPLC/PDA - Shimadzu®) using an analytic column C-18 (Phenomenex 150 mm × 4.6 mm, 10 μm), eluted with 50 mM phosphate buffer (pH 7.0): methanol (65:35, v/v) at 1 mL/min and detected spectrophotometrically at 532 nm. MDA levels were expressed as nmol of MDA/mg protein.

Protein and hemoglobin contents were determined by Bradford and Doles® reagents, respectively.

Determination of enzymatic activity

Determination of GPx activity was performed using the procedure described by Flohé & Günzler [43]. Tert-butylhydroperoxide was used as substrate, and the formation of oxidized glutathione (GSSG) was indirectly monitored spectrophotometrically through NADPH consumption at 340 nm (Power Wave x 340, Bio-Tek Instruments INC, software KC4 v3.0) for 3 minutes.

GR activity assay was performed according to Carlberg & Mannervik [44]. The reduction of GSSG to reduced glutathione (GSH) was measured through NADPH consumption and monitored spectrophotometrically at 37 °C for 20 min at 340 nm (Power Wave x 340, Bio-Tek Instruments INC, software KC4 v3.0). The balance of GPx and GR activities is crucial to maintain GSH levels, the most important non-enzymatic antioxidant and the substrate of two important antioxidant enzymes. As GSH measurements in erythrocytes can be compromised by methodological artifacts, such as a quick oxidation to GSSG [45, 46], GR/GPx activity ratio was calculated to better assess regulation of the GSH cycle.

GST activity assay was conducted by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione [47]. The formation of the complex was monitored at 25 °C for 15 min at 340 nm in a spectrophotometer (Power Wave x 340, Bio-Tek Instruments INC, software KC4 v3.0).

Catalase activity assay was evaluated by measuring the consumption of hydrogen peroxide [48]. The decrease in absorbance was monitored at 25 °C for 30 s at 240 nm in a spectrophotometer (Biochrom).

All enzyme activity was corrected by hemoglobin content and expressed as U/g of hemoglobin. All enzymatic assays were conducted in triplicate.

Statistical analysis

All variables showed homogenous variance on Bartlett’s test. Data were analyzed by one-way analyses of variance (ANOVA) followed by Newman-Keuls post-hoc test. Given that the total sample was predominantly female, the variable gender was entered as a factor in the ANOVA but evidenced no effect or interaction with other independent variables (0.202 ≤ p ≤ 0.989), and was therefore removed from the model.

Pearson’s correlation test was used to assess the correlation between GR/GPx ratio and MDA levels, and between MMSE and MDA and GR/GPx ratios in each group. As our hypothesis was that patients with cognitive deficit had lower antioxidant defenses and higher MDA production, a one-tailed analysis was used. The level of significance was set at 5% (p < 0.05, 95% confidence interval) and the statistical calculations were performed with SPSS Statistics software for Windows (version 14.0).
RESULTS
Socio-demographic characteristics and cognitive performance

Demographic data are shown in Table 1. The three groups did not differ regarding gender and age. However, MCI subjects had a lower educational level compared to that of healthy controls ($p = 0.006$). No difference was observed between AD and control or AD and MCI. Given that groups differed for schooling, this variable was included as a covariate in the proceeding analysis and removed when no significant effect was observed.

AD patients performed significantly worse on the MMSE than did MCI subjects and healthy elderly controls ($p < 0.001$). The MCI subjects also scored lower on the MMSE compared to controls ($p < 0.001$).

MDA levels in plasma

Average MDA levels measured in plasma of healthy aged controls, MCI, and AD patients were 38.8 ± 4.3, 56.4 ± 6.4, and 77.8 ± 8.6 nmol/mg of protein (Mean ± SEM), respectively (Fig. 1). Comparison with healthy aged controls, revealed significantly higher MDA levels in MCI (45%) [F(2,71) = 8.61, $p < 0.05$] and AD patients (105%) [F(2,71) = 8.61, $p < 0.001$]. AD patients also had higher mean MDA levels compared to MCI patients (38%) [F(2,71) = 8.61, $p < 0.05$].

Catalase activity in erythrocytes

Average catalase activity measured in erythrocytes of healthy aged controls, MCI and AD patients was 2.8 ± 0.1, 2.9 ± 0.1 and 3.3 ± 0.1 kU/g of hemoglobin (Mean ± SEM), respectively (Fig. 2). Comparison to healthy aged controls revealed a significantly higher catalase average activity in AD patients (18%) [F(2,87) = 5.66, $p < 0.01$]. AD patients also showed higher mean catalase activity compared to MCI patients (14%) [F(2,87) = 5.66, $p < 0.01$]. No difference in catalase average activity was found between healthy aged controls and MCI patients [F(2,87) = 5.66, $p > 0.05$].

Table 1
Demographic characteristics and cognitive performance in AD, MCI and control subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 26) or Mean (± SD)</th>
<th>MCI (n = 33) or Mean (± SD)</th>
<th>AD (n = 29) or Mean (± SD)</th>
<th>p (value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
<td>20.0 (76.9)</td>
<td>22.0 (66.7)</td>
<td>37.0 (67.3)</td>
<td>0.630 a</td>
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<tr>
<td>Age (years)</td>
<td>73.5 ± 5.8</td>
<td>76.8 ± 5.9</td>
<td>76.4 ± 5.2</td>
<td>0.072 b</td>
</tr>
<tr>
<td>Educational level (years)</td>
<td>9.4 ± 4.8</td>
<td>5.6 ± 3.9</td>
<td>7.9 ± 4.6</td>
<td>0.008*</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.1 ± 1.7</td>
<td>24.4 ± 2.4</td>
<td>21.1 ± 2.1</td>
<td>&lt; 0.001 b</td>
</tr>
</tbody>
</table>

MMSE = Mini Mental State Examination; *Value of less than 0.05 indicates significance; aChi square test, bANOV A with Newman-Keuls post-hoc test.
GPx, GR activity, and GR/GPx ratio in erythrocytes

Average GPx content measured in erythrocytes of healthy aged controls, MCI and AD patients was $57.8 \pm 4.4$, $61.5 \pm 3.4$ and $96.9 \pm 5.1$ U/g of hemoglobin (Mean ± SEM), respectively. AD patients showed significantly higher mean GPx activity compared to healthy aged controls, (68%) $[F(2,85) = 24.64, p < 0.001]$, and to MCI patients (58%) $[F(2,85) = 24.64, p < 0.001]$. No difference in mean GPx activity was detected between healthy aged controls and MCI patients $[F(2,85) = 24.64, p > 0.05]$.

Average GR content measured in erythrocytes of healthy aged controls, MCI and AD patients was $6.5 \pm 0.4$, $4.9 \pm 0.3$ and $5.3 \pm 0.2$ U/g of hemoglobin (Mean ± SEM), respectively. Comparison to healthy aged controls revealed significantly lower mean GR activity in AD patients (19%) $[F(2,85) = 6.99, p < 0.05]$ and in MCI patients (25%) $[F(2,85) = 6.99, p < 0.01]$. No difference in average GR activity was found between MCI and AD patients $[F(2,85) = 6.99, p > 0.05]$.

The ratio of GR/GPx activity was calculated in order to better evaluate the glutathione cycle. Average GR/GPx activity ratio in healthy aged controls, MCI and AD patients was $0.12 \pm 0.01$, $0.085 \pm 0.006$, and $0.059 \pm 0.002$ (Mean ± SEM), respectively (Fig. 3). In comparison to healthy aged controls, a significantly lower GR/GPx activity ratio was detected in MCI (29%) $[F(2,86) = 17.48, p < 0.001]$ and AD patients (51%) $[F(2,86) = 17.48, p < 0.001]$. AD patients also showed lower GR/GPx activity ratio compared to MCI patients (31%) $[F(2,86) = 17.48, p < 0.05]$.

GST activity in erythrocytes

Average GST activity measured in erythrocytes of healthy aged controls, MCI and AD patients was of $3.0 \pm 0.1$, $3.2 \pm 0.2$ and $3.0 \pm 0.2$ U/g of hemoglobin (Mean ± SEM), respectively (Fig. 4). No differences in GST activity were detected among AD, MCI and healthy controls $[F(2,83) = 0.31, p > 0.05]$.

Oxidative stress, antioxidant enzymes and cognitive performance

In the AD group, MDA levels were negatively correlated with GR/GPx ratio ($r = -0.364, p = 0.009$). Thus, high MDA levels were associated with low antioxidant defenses in AD patients. However, no significant correlation was observed in MCI and healthy elderly controls ($p > 0.10$). In the AD group, MMSE was negatively correlated with MDA levels. By contrast, a positive correlation between MMSE and GR/GPx ratio was observed in the same group. No significant association ($p > 0.10$) was observed between MMSE, MDA levels and GR/GPx ratios for the MCI and control groups (Table 2).

![Fig. 3. Mean GR/GPx activity ratio of healthy aged controls, MCI and AD patients. Data expressed as mean ± SEM. ***$p < 0.001$ – versus healthy aged controls and # $p < 0.05$ – versus MCI (one-way ANOVA followed by Newman-Keuls post-hoc test).](image-url)

![Fig. 4. GST activity in erythrocytes of healthy aged controls, MCI and AD. One-way ANOVA followed by Newman-Keuls post-hoc test. Data expressed as mean ± SEM. Hb = hemoglobin.](image-url)

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation between MMSE score, MDA levels and GR/GPx ratio in AD, MCI and control subjects</th>
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<tbody>
<tr>
<td>MMSE vs AD</td>
<td>AD MCI Control</td>
</tr>
<tr>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>MDA levels $-0.310$</td>
<td>$0.028^{*}$</td>
</tr>
<tr>
<td>GR/GPx ratio</td>
<td>$0.669$</td>
</tr>
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</table>

*Value of less than 0.05 indicates significance. Pearson’s coefficient.
DISCUSSION

The current findings suggest increased peripheral oxidative stress markers and reduced antioxidant defenses in AD and MCI compared to healthy aged individuals. A steady increase in plasma MDA levels and reduction in erythrocyte GR/GPx ratio were observed from healthy elderly controls to MCI and AD patients. Moreover, we also found an association between cognitive performance, oxidative stress and antioxidant defenses in AD patients, showing that low cognitive performance was associated with both elevated MDA levels and decreased GR/GPx ratio in AD patients. Animal and human studies have shown that oxidative stress markers precede the appearance of AD neuroanatomical hallmarks [12]. In fact, previous findings have shown that Aβ deposition induces lipid peroxidation [13, 49–51] in MCI and AD, indicating that oxidative stress may play a role in the neurodegenerative mechanism of AD [13, 52, 53]. Corroborating these findings, we have shown a steady increase in plasma MDA levels from healthy aged to MCI and mild AD subjects. MCI subjects presented intermediate levels of MDA compared to control and AD groups. In line with this finding, other authors have reported that elevated MDA level was the main consistent finding among peripheral markers of oxidative stress in AD [24–26, 33, 34, 54–57]. However, studies evaluating MCI and AD have failed to yield significant differences in MDA levels between MCI and AD subjects [33, 34]. Thus, the current results suggest that lipid peroxidation may be present early in the neurodegenerative process of dementia and may also differentiate very mild to mild forms of cognitive age-related disorders.

Antioxidant enzymes are crucial to maintain antioxidants within physiological levels. An imbalance in these enzymes can cause the accumulation of reactive species leading to cell damage. For instance, the free radical O2·− undergoes dismutation by superoxide dismutase (SOD) which generates H2O2; an inorganic peroxide, that can be removed by both GPx and catalase. While catalase is responsible for its direct decomposition to H2O and O2, H2O2 removal by GPx depends on GSH oxidation to GSSG, which is reduced back to GSH by GR. Moreover, GPx is also responsible for the removal of organic peroxides, although catalase is unable to achieve this. GSH is also involved in the conjugation of endogenous substances as well as xenobiotics which are catalyzed by GST [40].

The current study has found an imbalance of antioxidant defenses in AD and MCI subjects. Elevated GPx and catalase activities were observed in AD patients, compared to MCI and control groups. However, GR activity was similar in AD and MCI patients, yet both groups showed decreased GR activity compared to healthy aged individuals. Given that GPx and catalase enzymes are necessary to remove H2O2, the current findings suggest that increased activity of these enzymes may be a compensatory effect for the overproduction of H2O2 and lipid peroxides in AD subjects. Increased production of these peroxides has been proposed in our previous study in AD [25] and in reports by others in both AD [33, 35, 55, 58] and MCI [14, 33–35].

This is the first study of its kind to evaluate catalase in erythrocytes of MCI patients. No consensus was found with respect to its activity in AD. An increase in catalase activity was also reported [59, 60], however, others [55, 61] described decreased activity. Regarding GPx, an increase in its activity was also observed in AD patients in several studies [26, 57, 59, 62], however, some groups have reported a decrease in GPx activity both in AD and MCI [14, 33, 35, 55], while others evaluating AD alone observed no difference [54, 63].

While we report an impairment in GR activity both in MCI and AD, lower GR activity has previously been demonstrated in AD alone [55, 57]. However, other groups failed to observe changes in GR activity in MCI [26, 34] or AD [26, 34, 62]. Our results suggest early impairment in the production of antioxidant defenses such as GSH, which could imply a deficient antioxidant cell response [64]. In fact, the steady reduction of GPx/GR ratio observed in our study suggests that the reduced capacity to recycle GSH in MCI and AD is due to an imbalance in the enzymatic activities of the GSH cycle. By the same reasoning, the decrease in GSH/GSSG ratio in erythrocytes of both MCI and AD patients described by Bermejo et al. [14] further corroborates our result of lower GR/GPx ratio. The negative correlation between MDA levels and GR/GPx ratio observed in AD patients is in agreement with the interpretation that the lower the antioxidant enzyme capacity, the higher the MDA levels. Taken together, these findings suggest that MCI and AD subjects could be more vulnerable to the effects of oxidative stress since their antioxidant defenses may be deficient, leading to an impaired cell response. Thus, it may also be proposed that this deficient production of antioxidants defenses pre-exists in MCI.

Given that oxidative stress may play a role in the neurodegenerative process of dementia, the present study also sought to ascertain whether lipid peroxidation and antioxidant defenses could be associated...
with cognitive impairment. We found a negative correlation between MMSE scores and MDA levels in AD patients and a positive correlation between MMSE and GR/GPx ratio in the AD group, thus demonstrating that lower cognitive performance was associated with lower antioxidant defenses. The lack of association between cognitive performance, MDA levels and GR/GPx in MCI subjects may reflect an adaptive stage of body response, whereas the significant correlations between cognition, oxidants and antioxidants enzymes activity may reveal a disruptive stage, observed only in AD patients.

A large body of evidence suggests that amnestic MCI is an intermediate state between normal aging and AD [1]. Based on brain oxidative stress markers of individuals with cognitive impairment, Sultana & Butterfield [51] suggested that oxidative stress could be an early event in the progression of MCI to AD. Baldeiras et al. [34] evaluated peripheral levels of a broad spectrum of enzymatic and non-enzymatic antioxidants and also suggested that the oxidative alterations seen in AD patients were already present in MCI patients AD patients. Corroborating these findings, our results suggest that MCI is not only a clinical transition state of dementia, but also may represent an early biochemical stage of the neurodegeneration process.

The presence of the ε4 allele in apolipoprotein E (APOE) gene has been recognized as a major risk for sporadic AD [65-67]. There are several hypotheses about the role of ε4 allele of APOE in AD physiopathology. The ε4 gene product might: (a) interfere with Aβ deposition in brain tissue [68, 69]; (b) have a regulatory role in neuronal tau protein metabolism [70]; (c) act in a negative way on the cholinergic system [71]; (d) produce a less efficient antioxidant effect [72]; and (e) be associated with an increased LDL cholesterol level [73]. A few studies investigated the relationship between APOE genotype and peripheral markers of oxidative damage. Ihara et al. [74] showed that APOE 4 AD patients had higher blood hydroxyl radical levels than those without this allele or non-demented subjects. They did not detect any correlation between erythrocyte SOD activity and protein levels of CuZn-SOD and APOE genotype. Fernandes and colleagues [75] did not find a correlation between free intracellular Ca2+ concentration in platelets and APOE genotype of AD patients and matched controls. The same group [76] did not establish a clear association between blood redox status and allele ε4 status in AD patients and controls. Kinoshita et al. [77] also did not demonstrate any influence of APOE genotype on peripheral antioxidant status. On the other hand, Aybek et al. [78] showed that AD patients with at least one ε4 allele had higher MDA levels than controls. In our previous study [79], we described an association between ε4 allele carriers and nitric oxide synthase activity, however, we did not find any association with the thioarbituric acid reactive substances (TBARS). Baldeiras and co-workers (2008) demonstrated that the presence of ε4 of APOE was associated with a decrease in GSH and increase in MDA levels both in MCI and mild AD patients. It would be interesting to evaluate if MCI and AD patients with ε4 allele of APOE would also have a decrease in GR/GPx ratio.

It is important to point out that the inconsistencies found between previous studies and the current findings may be due to differences in clinical inclusion criteria and laboratory methodology [81]. Regarding inclusion criteria, some studies did not specify which sub-group of MCI was being evaluated while others included AD patients at different stages of the disease. In order to achieve a more homogeneous sample, the present study included only amnestic MCI patients and mild AD patients. Moreover, studies evaluating peripheral antioxidant enzyme capacity and lipid peroxidation markers were conducted in different matrices, such as erythrocytes, plasma and serum, hampering any comparisons among them.

In conclusion, elevated production of lipid peroxidation marker, in addition to reduced enzymatic antioxidant defenses, were observed over the course of normal to pathological cognitive aging. Both MDA levels and GR/GPx ratio differentiated AD patients from healthy elderly controls, confirming MCI as an intermediate stage between normal cognitive aging and dementia in terms of biochemical markers. Although an imbalance between prooxidant and antioxidant defenses was evidenced in the MCI subjects, poor cognitive performance was associated with both elevated lipid peroxidation and decreased enzymatic antioxidant defenses only in AD patients.

ACKNOWLEDGMENTS

The authors would like to thank Fernando Kok for helpful comments. This research was supported by a grant to T.M. from the Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP (2004/10205-0). L.L.T., N.B.Q., R.T.G., W.L.M. and J.N.S.T were supported by FAPESP. A.P.M.L. and S.B.B. are research fellows of CNPq, Brazil. Authors’ disclosures available online (http://www.j-alz.com/disclosures/view.php?id=826).


