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Oxidative balance, homocysteine, and uric acid levels in older patients with Late Onset Alzheimer's Disease or Vascular Dementia

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ABSTRACT

This study aimed to investigate whether Late Onset Alzheimer's Disease (LOAD) and Vascular Dementia (VAD) might be associated with a distinct profile of oxidative stress (OxS) peripheral markers. Serum levels of hydroperoxides, homocysteine, advanced oxidation protein products, uric acid, thiols, and total and residual antioxidant power were assessed in 103 mild cognitive impairment (MCI), 89 LOAD, 54 VAD patients and 48 Controls. Compared with Controls, a similar oxidative unbalance (high hydroperoxides and low residual antioxidant power) was observed in MCI, LOAD and, although less pronounced, VAD. Moreover, individuals with simultaneously high levels of homocysteine and uric acid, both well-known risk factors for cardiovascular disease, had a high probability to be affected by VAD (O.R.:10.50; 95% C.I.: 2.33–47.2), but not LOAD (O.R.: 3.0; 95% C.I.:0.86–10.76) compared with individuals with normal values. Our data suggest that, although they might share a common OxS-related pathogenesis, VAD and LOAD might maintain some distinctive features, with a predominance of "vascular component" in VAD compared with LOAD.

1. Introduction

Late Onset Alzheimer's Disease (LOAD) and Vascular Dementia (VAD) represent the two most common forms of dementia in elderly populations with nearly 70% of patients belonging to LOAD and 20% to VAD [1]. Thus far, the clear cut-off diagnosis differentiating LOAD and VAD is complicated by the lack of well-recognized discriminatory parameters, in particular as regards the cognitive profile [2]. Moreover, it is acknowledged that some markers of vascular injury typical of VAD might coexist with the diagnosis of LOAD [3,4]. In parallel, amyloid β (Aβ) accumulation, one of the recognized pathological hallmarks of Alzheimer disease, has been found, at a minor extent, also in patients affected by VAD [5].

It has been proposed that oxidative stress (OxS) might play a key-role in the pathogenesis of both LOAD and VAD [4]. Indeed, free radicals, including reactive oxygen species (ROS), can react with substrates essential for the survival of neurons such as proteins, lipids, and nucleic acid, leading to neuropathological lesions and to brain damage [6,7]. Besides, brain is highly susceptible to oxidative damage, mainly because of the high level of polyunsaturated fatty acid (the main target of free radicals attack), the high oxygen requirement for its metabolic processes, and the low concentration of antioxidants contrasting ROS activity [8,9]. In LOAD, OxS might be both the cause of Aβ accumulation, by increasing Aβ precursor protein (APP), and the effect of Aβ accumulation, thus emphasizing the neurotoxicity of the amyloid [10–12]. On the other hand, the origin of OxS in VAD might be more associated to the typical vascular abnormalities observed in patients affected by this type of dementia. Indeed, vascular endothelium is able to synthesize, store, and release free radicals in response to stimuli such as injury and hypoxia/hypoperfusion [13]. Vascular OxS is also implicated in the onset of several well-recognized risk factors for VAD (and LOAD) including diabetes, stroke, atherosclerosis, and hypertension [13].

The relationship between OxS and dementia development has been nicely characterized in animals, in vitro, and in post-mortem models, but it still awaits confirmations by studies on living human patients [14–18]. This is more evident for clinical data regarding VAD, which are substantially contrasting since both increased markers of oxidative damage and/or decrease antioxidants [19–21] or normal OxS peripheral level [22] have been reported. These discrepancies might be explained by some limitations of previous studies including small sample size, lack of control over relevant confounding factors (i.e. age, diabetes, and hypertension), and use of OxS marker detection methods with low reliability.

In this study we tried to overcome some of these limitations. To address this issue, we evaluated a number of distinct serum indicators

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of OxS in a large sample of 294 elderly individuals, including patients affected by VAD, LOAD, mild cognitive impairment (MCI), and cognitively healthy Controls.

2. Material and methods

2.1. Study design

Two hundred ninety four subjects were enrolled into the study from 2006 to 2012, including:

1. Eighty nine elderly patients with diagnosis of LOAD by the NINCDS–ADRDA criteria [23] (mean age: 77.8 years) referring to the Day Service for Cognitive Decline (University of Ferrara, Italy). Only patients with "probable" Alzheimer's disease were included in order to increase specificity. The Global Deterioration Scale ranged from stage 4 to stage 6.
2. Fifty four elderly patients with diagnosis of VAD by the NINDS–AIREN criteria [24] (mean age: 77.8 years) referring to the same Day Service. Only patients with "probable" VAD were enrolled. The Global Deterioration Scale ranged from stage 4 to stage 6.
3. One hundred three elderly patients with diagnosis of MCI referring to the same Day Service (mean age: 78.8 years). MCI was defined as the presence of short/long-term memory impairment, with/without impairment in other single or multiple cognitive domains, in an individual who didn't meet the standardized criteria for dementia [25]. We also required that the patient with MCI would be still independent in the activities of daily living (ADLs). The vast majority of these individuals were affected by amnestic multi-domain MCI. Subjects with MCI possibly related with known causes (e.g. severe depression, severe vitamin B_{12} deficiency) had been excluded. The diagnosis of dementia or MCI was made by trained geriatricians.
4. Forty eight healthy elderly individuals (Controls) without evidence of dementia and without any functional disability attributable to cognitive impairment (mean age: 79.3 years). Only individuals $\geq$70 years of age were enrolled to obtain a sample with a mean age similar to the other three groups.

This study was carried out in accord to the Declaration of Helsinki (World Medical Association, http://www.wma.net), the guidelines for Good Clinical Practice (European Medicines Agency, http://www.ema.europa.eu) and it was approved by the local Ethic Committee. Written informed consent was obtained from each subject (and/or their caregiver if demented) during the first visit.

Personal data and medical history were collected by a structured interview from patients and caregivers. All patients underwent a general and neurological examination. For neuropsychological assessment, all patients were given a battery of tests as previously described [26]. Routine analyses including liver function, serum folate and B_{12} vitamin, thyroid function, blood cell count, and arterial oxygen saturation were performed to exclude causes of secondary cognitive impairment. Subjects affected by, severe congestive heart failure, severe liver or kidney disease, cancer, and chronic obstructive pulmonary disease were not included in the study. There were no evidences of acute illnesses at the time of clinical observation and blood sampling; no subject was taking NSAIDS, antibiotics or steroids at the time of recruitment. Criteria used for the diagnosis of diabetes, hypertension and cardiovascular diseases (CVD), and to define smoker category (>180 packs/year) were previously described [26].

2.2. Assays of biochemical parameters

Venous blood was collected from subjects upon an overnight fast. Blood was centrifuged at 3000 RPM for 10 min and serum was stored at −80 °C until analysis.

Hydroperoxides were assessed by colorimetric assay based on the reaction between these lipid peroxidation by-products and N, N-diethyl-para-phenylendiamine [27,28]. This assay is based on the ability of transition metals to catalyze the formation of free radicals with high reactivity, which strongly interact with the chromogenic reagent (absorption peak at 505 nm). Results were expressed as Carr Units (CU) where 1 CU corresponds to 0.023 mM of H_2O_2. The intra-assay coefficient of variation (CV) was 2.5%, whereas the inter-assay CV was 3.5%. The limit of detection was 40 CU.

Total antioxidant power (TAP) was assayed in serum accordingly to original description by Benzie and Strain [29]. This method, which is widely known as FRAP (Ferric Reduction Antioxidant Power), measures the ability of serum antioxidants (such as uric acid, ascorbic acid, α-tocopherol) to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ) that absorbs at 593 nm. The intra-assay CV was 3.9%, whereas the inter-assay CV was 9.9%. The limit of detection was 10 FRAP units.

The concentration of serum uric acid (μmol/L) was determined by the direct enzymatic method [30] in which uric acid was oxidized by uricase coupled with peroxidase, and the results were measured colorimetrically. The intra-assay CV was 2.5%, whereas the inter-assay CV was 5.3%. The limit of detection was 18 μmol/L. FRAP assay is strongly influenced by the amount of uric acid, of which role as physiological antioxidant is highly controversial [31]. To overcome this bias, uric acid contribution in FRAP assay was subtracted from TAP values of total FRAP value. The resulting parameter (still expressed in FRAP units), i.e. residual antioxidant power (RAP), affords a reliable index of antioxidant status in uric acid-rich fluids such as serum [31].

The concentration of serum thiols (μmol/L) was evaluated by the colorimetric (detection at 412 nm by spectrophotometer) 5′5-dithiobis-(2-nitrobenzoic acid)-based assay [32]. The intra-assay CV was 6.5%, whereas the inter-assay CV was 8.5%. The limit of detection was 20 μmol/L.

Advanced oxidation protein products (AOPP) were assessed by spectrophotometric detection (at 340 nm) according to Capeillère-Blandin [33]. Concentrations of AOPP, determined in reference to the calibration curve, were expressed in μmol/L. The intra-assay CV was 5.1%, whereas the inter-assay CV was 9.5%.

Homocysteine level was determined by the Liquid Stable (LS) 2-Part Homocysteine Reagent (Axis-Shield Diagnostics Ltd., UK) using a ROCHE COBAS INTEGRA 800 chemistry analyzer following the manufacturer's instructions. Concentrations of homocysteine, determined in reference to the calibration curve, were expressed in μmol/L. The intra-assay CV was 1.5%, whereas the inter-assay CV was 2.6%.

2.3. Brain computer tomography scan

All patients (LOAD and MCI) underwent a brain Computer Tomography (CT). The instrument used was a third generation SIEMENS SOMATOM HQ. The slice thickness was 10 mm. The CT scan data were used to support the clinical diagnosis.

2.4. Statistical analysis

Means were compared by ANOVA and ANCOVA (Fisher's least significant difference as *post-hoc* test); in ANCOVA analysis, CVD (yes/no), hypertension (yes/no), diabetes (yes/no), current smoking (yes/no), and sex (M/F) were included as covariates. Prevalences were compared by the χ^2 test. Since the distribution of hydroperoxides, RAP, thiols, and homocysteine were skewed, the values were log-transformed in order to approximate a normal distribution before entering univariate and multivariate analysis.

The risk of receiving a diagnosis of LOAD or VAD (Odds Ratio, O.R.; 95% confidence interval, C.I.—95%) in subjects with hyperuricemia or hyperhomocysteinemia was calculated by multivariate logistic regression analysis adjusting for CVD, hypertension, diabetes, current smoking (yes/no), and sex. High/low uric acid (UA) and homocysteine (HOMO) plasma levels were also combined into 3 groups in order to evaluate the risk for LOAD and VAD associated with the combination of these

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