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PON-1 and ferroxidase activities in older patients with mild cognitive impairment, late onset Alzheimer's disease or vascular dementia

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Abstract

Background: A large body of evidence suggests that not only cerebral but also systemic oxidative stress (OxS) might be involved in the pathogenesis of late onset Alzheimer's disease (LOAD) and vascular dementia (VAD), as well as of the prodromal phase of dementia, the so-called mild cognitive impairment (MCI). In the present study, we evaluated whether paraoxonase 1 (PON-1) and ferroxidase (FeOx) activities, because of their well acknowledged effectiveness as systemic antioxidants, might be associated with dementia and/or MCI.
Methods: Serum arylesterase and paraoxonase of PON-1, along with FeOx I (ceruloplasmin-related) and II activities were assessed in 223 MCI, 162 LOAD, 65 VAD patients, and in 143 older normal cognitive controls.
Results: Among the enzymatic activities examined, only arylesterase significantly changed across the groups (ANOVA: $p<0.001$), with similar lower levels in MCI, LOAD, and VAD compared to controls. By multivariate logistic regression analysis we showed that, in respect to controls, low levels (under the median value) of serum arylesterase were independently associated with an increase in the likelihood of being affected by LOAD [odds ratio (OR) 2.8, 95% confidence interval (CI) 1.5–5.0], VAD (OR 2.7, 95% CI 1.2–6.2), or MCI (OR 2.3, 95% CI 1.3–3.8).
Conclusions: Overall, our results suggest that depression of PON-1, and in particular, of arylesterase activity, in serum might be an early feature of dementia-related diseases. Further longitudinal exploration of the role of this enzyme in the onset and progression of these disorders are required.

Keywords: Alzheimer's disease; ferroxidase; mild cognitive impairment; oxidative stress; paraoxonase-1; vascular dementia.

Introduction

The physiopathological mechanisms underlying the two most common causes of dementia in elderly, i.e., late onset Alzheimer's disease (LOAD) and vascular dementia (VAD), have not been fully elucidated yet. Relevant to this regard, mounting evidence suggests that oxidative stress (OxS), low-grade inflammation and iron dyshomeostasis might synergically act as pathogenic mediators of these disorders [1–4]. It is well documented that the atherogenicity of LDL sharply increases upon oxidative modification induced by high levels of reactive oxygen species (ROS) [5]. Atherosclerosis, and in general all pathological conditions leading to blood vessels damage, are recognized risk factors for both cerebrovascular disease and LOAD [6]. Vascular lesions are, in turn, sources of OxS since, in response to stimuli, such as injury and hypoxia, vascular endothelium, can synthesize, store, and release free radicals [7, 8]. A similar detrimental self-perpetuating cycle occurs in systemic inflammatory processes which seem to underline both VAD and LOAD [2, 9]. Excessive accumulation of oxidative damage against biomolecules could also originate in vivo from metal-catalyzed Fenton reaction, which involves free ferrous ion and leads to the formation of the most potent ROS, hydroxyl radical (·OH), both in extracellular fluids and intracellular environment

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[10, 11]. Accumulation of iron, observed in cerebrospinal fluid (CSF), basal ganglia and hippocampus [4, 11], along with the well documented pro-oxidant ability of amyloid β (Aβ) soluble aggregates [12] and nerufibrillary tangles [13], were all potential triggers of ROS production in LOAD brain.

Recent findings of systemic alterations in Redox balance of LOAD and VAD patients [14–17] emphasize the roles of molecules implicated in the maintenance of a correct homeostasis between circulatory oxidants and antioxidants. Paraoxonase 1 (PON-1) (E.C. 3.1.8.1) and ceruloplasmin (Cp) (E.C. 1.16.3.1) enzymatic activities represent two of the most valuable examples in this frame.

The catalytic activity of PON-1, which is present in human plasma on the surface of high density lipoprotein (HDL), include paraoxonase, arylesterase, and lactonase which catalyze the hydrolysis of organophosphates, aromatic esters and lactones, respectively [18, 19]. A large body of evidence suggest that, both paraoxonase and arylesterase activity of this protein are implicated in the anti-inflammatory and antioxidant activities of HDLs [20, 21]. Indeed, PON-1 is able to prevent LDL oxidation in vitro [18], and this potential anti-atherosclerotic function could account for the association between low level of its serum activity and high risk of adverse cardiovascular events [20–22].

Potentially, keeping free Fe^{2+} levels low in blood is a very effective way to contrast the generation of ROS [23]. This preventive action is prevalently afforded by the ferroxidase (FeOx) enzyme activity of Cp (FeOx I) and, to a less extent (10%–20% of the total serum FeOx), by a second copper-containing non-ceruloplasmin iron oxidase, FeOx II [24]. Cp is a multi-functional, copper protein of liver origin, which plays also a prominent role in iron metabolism since, by catalyzing oxidation of Fe^{2+} to Fe^{3+}, it promotes iron loading into apo-transferrin and its efflux from the liver to the other organs and tissues [25].

Given the aforementioned multiple roles of PON-1 and Cp-related FeOx activity, it is conceivable to hypothesize that an alteration of these two enzymes might be associated with the risk of developing dementia in older population. Nevertheless, this thesis has not been definitely confirmed by solid epidemiological data, mainly because of important methodological limitations (i.e., small sample size, lack of consideration of important confounding factors, etc.) affecting the available current literature [25–30]. In the attempt to shed light upon this still confusing and uncertain scenario, we conducted the present study dealing with the evaluation of serum paraoxonase, arylesterse, and FeOXs activity in a large sample of older individuals (n=593) including patients affected by VAD, LOAD, mild cognitive impairment (MCI), and cognitively healthy controls.

Materials and methods

Study design

A total of 593 older subjects referring to the Day Service for Cognitive Decline (University of Ferrara, Italy) were enrolled into the study from 2007 to 2013. The study sample included:

162 patients with diagnosis of LOAD according to NINCDS-ADRDA criteria [31]. Only patients with 'probable' Alzheimer's disease were included in order to increase specificity. The Global Deterioration Scale (GDS) ranged from stage 4 to 6.

In total 65 elderly patients with diagnosis of VAD by the NINDS-AIREN criteria [32] with GDS between stages 4–6.

There were 223 patients with MCI. This condition 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 did not meet the standardized criteria for dementia [33]. We also required that the patient with MCI would be still independent in the activities of daily living. Most of these individuals were affected by amnestic multi-domain MCI. Subjects with MCI possibly related with known causes (e.g., severe vitamin B12 deficiency, severe depression etc.) were excluded.

There were 143 individuals (controls) without symptoms of dementia and without any functional disability attributable to cognitive problem.

This study was conducted accordingly to the Declaration of Helsinki (World Medical Association, http://www.wma.net), and the guidelines for Good Clinical Practice (European Medicines Agency, http://www.ema.europa.eu). Written informed consent was obtained from each subject (and/or their caregiver if demented) prior to the inclusion in the research protocol.

Personal data and anamnesis were collected by a structured interview from patients and caregivers. The diagnosis of dementia or MCI was made by trained geriatricians. All patients underwent a general and neurological examination as well as neuropsychological assessment by a battery of tests, as previously described [2]. In particular, mini mental state examination (MMSE) was used as tool to evaluate the possible presence, and the degree of cognitive impairment [scores ≤27 points (out of 30) indicates a normal cognition]. Moreover, patients, to support the initial clinical diagnosis of MCI, LOAD or VAD, underwent a brain computer tomography (by Siemens Somaton HQ). Routine analyses including liver function, serum folate and B12 vitamin, thyroid function, blood cell count, and arterial oxygen saturation were performed to exclude causes of secondary cognitive impairment. Patients with severe liver or kidney disease, severe congestive heart failure, cancer, and chronic obstructive pulmonary disease were excluded from the study. There was no evidence 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 disease (CVD), and to define smokers category (≤10 pack-year history of ever-smoking) were described elsewhere [2].

Assays of biochemical parameters

Venous blood was collected from subjects after an overnight fast. Blood was centrifuged at 4.650 *g* for 10 min and the resulting serum was stored at −80 °C until analysis.

Serum arylesterase and paraoxonase activity were independently assessed by UV-VIS spectrophotometry in a 96-well plate format (Tecan infinite M200 from Tecan group Ltd, Switzerland) using phenyl acetate and paraoxon (Sigma Aldrich, St Louis, MO, USA) as substrates, respectively [20, 34].

Briefly, initial hydrolysis rates of phenylacetate were assessed at 270 nm after adding 10 µL of sample to 240 µL of reactions mixtures composed of 1 mM phenylacetate, 9 mM Tris-HCl, pH 8, and 0.9 mM $CaCl_2$ (temperature: 24 °C). An extinction coefficient of 1310/M/cm was used for calculating units (KU) of arylesterase activity, which are expressed as the µmoles of phenyl acetate hydrolyzed per minute per liter of serum.

Paraoxonase activity assays was carried out at 24 °C by assessing (at 405 nm) the rate of formation of para-nitrophenol, upon adding 5 µL in 245 µL of reaction mixtures composed of 1.5 mM paraoxon, 10 mM Tris-HCl, pH 8, 0.9 M NaCl, and 2 mM $CaCl_2$. An extinction coefficient of 17000/M/cm was used for calculating units of paraoxonase activity, which are expressed as the nanomoles of para-nitrophenol generated per minute per milliliter of serum.

As previously reported, using this dual-substrate method to assess phenotype distribution, the occurrence of PON-1_{192} genotype can be accurately calculated [34]. The genetic polymorphism at codon Q192R is responsible for the presence of three phenotypes: QQ (wild type, low activity), QR (intermediate) and RR (high activity). The ratio of the paraoxonase activity, in the presence of 1 M of NaCl, to the arylesterase activity was used to assign individuals to one of the three possible phenotypes. Cut-off values between phenotypes were as follows: type QQ, ratio <2.95; type QR, ratio 2.95–6.91 and type RR, ratio >6.91.

Total FeOx activity was measured in serum samples according to Erel's method [24] with some minor modifications. Briefly, 5 µL of sample was added to 195 µL of acetate buffer (0.45 M, pH=5.8). After 1 min incubation at 37 °C, 43 µL of 370 mM $Fe(NH_4)_2SO_4$ was added, and the resulting mix was incubated for further 3.8 min at 37 °C. At the end of the incubation, 20 µL of chromogen (3-(2-Pyridyl)-5,6-bis-[2-(5-furylsulfonic acid)]-1,2,4-triazin) was added. The rate of formation of colored complex (formed by the chromogen and ferrous ions) was recorded at 600 nm. For FeOx II, the same procedure was applied, but using serum which was previously mixed with sodium azide, which instantly and completely inhibited FeOx activity of Cp. FeOx I activity was estimated from the difference between total FeOx and FeOx II. The amount of enzyme that converted 1 µmol of substrate into product per minute in 1 liter of sample was defined as 1 U/L.

Albumin, creatinine, high sensitivity C-reactive protein (Hs.CRP), hemoglobin, total cholesterol (TC), triglycerides, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) concentrations were measured by routine laboratory methods.

Statistical analysis

Mean values were compared by ANOVA (Fisher's least significant difference – LSD as post hoc test) or Kruskall-Wallis for variable with not normal distribution. Prevalence was compared by the χ^2-test. The risk of receiving a diagnosis of MCI, LOAD, or VAD (OR; 95% CI) in subjects with low values (below the median value=104 KU/L) of arylesterase was calculated by multivariate logistic regression analysis adjusting for age, CVD, hypertension, diabetes, current smoking (yes/no), gender, and HDL-C. A two-tailed probability value <0.05 was considered statistically significant. SPSS 17.00 for Windows (Chicago, IL, USA) was used for statistical analysis.

Results

In Table 1 are displayed the main characteristics of the subjects enrolled into the study. Individuals included in the control group were younger; MCI and VAD patients displayed a lower prevalence of female gender. Comorbidities (i.e., diabetes, hypertension, and CVD) were less frequent in non-demented controls compared to the other groups; in good agreement with current epidemiological literature, diabetes and CVD were more frequent in VAD compared with LOAD. With the exception of Hs.CRP (which was similarly higher in controls and VAD compared to the other groups), no significant differences emerged as regards the other biochemical parameters examined.

As shown in Table 2, serum arylesterase, but not paraoxonase activity of PON-1 was significantly (ANOVA: $p<0.001$) and similarly lower in MCI, LOAD, and VAD compared to controls. Conversely, the activity of either FeOx I or II did not show any significant change across the groups.

In order to check whether the decrease in serum arylesterase activity observed in MCI, LOAD and VAD was due only to aging itself, we stratified the sample in two subsamples of younger or older subjects (by using as age cut-off the value of 77 years, i.e., the median age of the sample). As shown by Table 3, arylesterase activity exhibited a similar trend in both groups, with lower level in all three groups of patients compared to controls (Kruskall-Wallis: $p<0.01$).

The distribution of PON-1_{192} phenotypes (QQ, QR, RR) was at the Hardy-Weinberg equilibrium in both total sample and each subsamples (Table 4). Notably, in control group the distribution of the three phenotypes was not significantly different from those observed in MCI, LOAD, or VAD groups. The same negative results emerged by comparing the phenotypic frequency between MCI and the two groups with demented patients. This result is not surprising since QQ, QR, and RR alloenzymes exhibit different hydrolysis rates using paraoxon as substrate, whereas their ability to hydrolyze phenylacetate is unmodified ([34] and Supplemental Material, Table 1, that accompanies the article http://www.degruyter.com/view/j/cclm.2015.53.issue-7/cclm-2014-0803/cclm-2014-0803.xml?format=INT).

Table 1 Main characteristics of older patients with late onset Alzheimer's disease (LOAD), vascular dementia (VAD), mild cognitive impairment (MCI), and non-demented controls.

	Controls (n=143)	MCI (n=223)	VAD (n=65)	LOAD (n=162)
Age, years[a]	68.8±9.3	76.4±6.3[b]	77.7±7.0[b]	78.6±5.6[b]
Gender, females, %[a]	76.9	60.0[b]	54.2[b]	70.4[c]
Education, years	8 (5–13)	5 (5–8)	5 (4–8)	5 (3–5)
MMSE score[e]	26.7 (25.2–28.3)	24.4 (22.2–26.7)	21.4 (18.7–23.4)	21.0 (18.4–23.7)
Hypertension, %[a]	47.1	58.1[b]	68.5[b]	63.5[b]
Smoking, %	7.1	8.8	8.5	6.0
Diabetes, %[a]	11.0	16.1[b]	32.1[b,c]	13.2[d]
CVD, %[a]	10.2	25.5[b]	35.6[b]	14.2[d]
Tot. Chol., mmol/L	5.4 (4.7–6.1)	5.3 (4.5–6.0)	5.1 (4.7–5.8)	5.4 (4.8–6.1)
Triglycerides, mmol/L	1.3±0.7	1.4±0.7	1.3±0.8	1.3±0.5
LDL-C, mmol/L	3.2 (2.6–3.8)	3.1 (2.6–3.6)	3.4 (2.5–4.4)	3.3 (2.8–4.2)
HDL-C, mmol/L	1.6±0.5	1.5±0.4	1.5±0.4	1.6±0.4
Hs.CRP, mg/L[e]	2.8 (1.0–5.9)	1.9 (1.0–4.7)	3.1 (1.1–6.1)	1.9 (0.8–4.0)
Hemoglobin, g/dL	13.7 (12.6–14.5)	13.4 (12.4–14.3)	13.3 (12.0–14.3)	13.1 (12.4–14.2)
Albumin, g/L	41.0 (38.2–43.2)	40.0 (38.3–43.4)	40.4 (38.9–43.7)	41.3(38.5–43.3)
Creatinine, μmol/L	79.2 (70.4–96.8)	79.2 (70.4–96.8)	96.8 (79.2–140.8)	79.2 (61.6–96.8)

Mean±standard deviation for normally distributed variables; median (interquartile range) for not normally distributed variables; percentage for discrete variables. [a]$p<0.05$ ANOVA or χ^2-test (post hoc test: [b]$p<0.05$ vs. Controls; [c]$p<0.05$ vs. MCI; [d] $p<0.05$ vs. VAD); [e]$p<0.05$ Kruskal-Wallis. CVD, cardiovascular disease; MMSE, Mini Mental State Examination.

Table 2 Serum paraoxonase/arylesterase and FeOx I/II activities in older patients with late onset Alzheimer's disease (LOAD), vascular dementia (VAD), mild cognitive impairment (MCI), and non-demented controls.

	Controls (n=143)	MCI (n=223)	VAD (n=65)	LOAD (n=162)
Paraoxonase activity, U/L	86 (48–163)	80 (46–151)	76 (39–124)	94 (49–150)
Arylesterase activity, KU/L[a]	100 (82–125)	88 (58–106)	83 (55–102)	86 (58–108)
FeOx I, U/L	492 (414–563)	482 (398–564)	451 (409–540)	500 (427–581)
FeOx II, U/L	105 (78–156)	118 (89–228)	136 (92–230)	117 (90–227)

Data presented as median (interquartile range), because not normally distributed. [a]Kruskal-Wallis $p<0.001$.

Table 3 Serum arylesterase activity in younger or older patients with late onset Alzheimer's disease (LOAD), vascular dementia (VAD), mild cognitive impairment (MCI), and non-demented controls.

	Controls (n=41)	Age >77 years MCI (n=114)	VAD (n=35)	LOAD (n=105)
Age, years	80.9±3.6	80.9±3.1	82.1±3.6	81.7±3.2
Arylesterase activity, KU/L[c]	101 (81–124)	86 (68–106)	84 (68–109)	86 (66–108)
	Controls (n=102)	**Age ≤77 years MCI (n=109)**	**VAD (n=30)**	**LOAD (n=57)**
Age, years[a]	65.8±6.6	71.0±4.7[b]	71.2±5.5[b]	72.1±3.3[b]
Arylesterase activity, KU/L[c]	104 (84–130)	88 (74–106)	86 (65–103)	82 (70–108)

Mean±standard deviation for normally distributed variables; median (interquartile range) for not normally distributed variables. 77 years=median age of total sample. [a]$p<0.05$ ANOVA (post hoc test: [b]$p<0.05$ vs. controls); [c] Kruskal-Wallis $p<0.01$.

Table 4 PON-1_{192} phenotype and allele frequencies in late onset Alzheimer's disease (LOAD), vascular dementia (VAD), mild cognitive impairment (MCI), and non-demented controls.

Groups	PON1 Phenotypes		
	QQ (n=388)	QR (n=192)	RR (n=13)
Controls	0.678 (97)	0.294 (42)	0.028 (4)
MCI	0.628 (140)	0.354 (79)	0.018 (4)
VAD	0.723 (47)	0.231 (15)	0.046 (3)
LOAD	0.642 (104)	0.346(56)	0.012 (2)

χ^2=1.71, p=0.425 for comparison of Controls vs. MCI; χ^2=1.21, p=0.545 for comparison of Controls vs. VAD; χ^2=1.73, p=0.420 for comparison of Controls vs. LOAD; χ^2=4.70, p=0.09 for comparison of MCI vs. VAD; χ^2=1.24, p=0.888 for comparison of MCI vs. LOAD; χ^2=4.82, p=0.089 for comparison of VAD vs. LOAD.

Multivariate logistic analysis was finally run to check whether decreased serum levels of arylesterase activity might affect the risk of receiving a diagnosis of MCI or either forms of dementia, regardless of potential confounding factors, such as age, gender, hypertension, CVD, diabetes, smoking status, and HDL-C. As shown in Figure 1, low levels of arylesterase were independently associated with higher likelihood of being affected by MCI (OR 2.3, 95% CI 1.3–3.8), LOAD (OR 2.8, 95% CI 1.5–5.0), and VAD (OR 2.7, 95% CI 1.2–6.2) compared to controls. Of interest, the multi-adjusted OR only slightly differed from those obtained in the respective univariate models (MCI, OR 2.5, 95% CI 1.6–4.0; LOAD, OR 2.6, 95% CI 1.5–4.3; VAD, OR 2.9, 95% CI 1.4–6.0); this observation suggests a negligible interference of potential confounders on the observed association (see Supplemental Material, Tables 2–4).

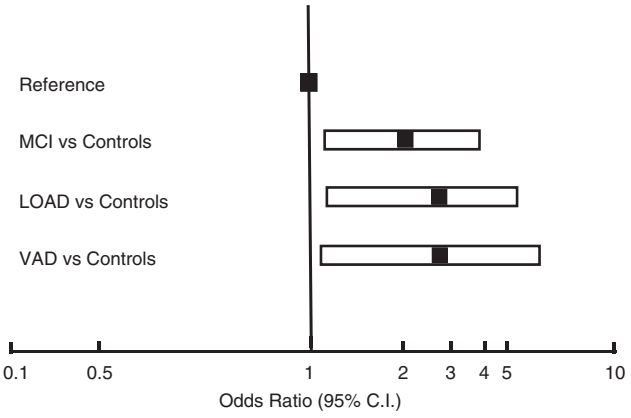

Figure 1 Multi adjusted odds ratio (95% confidence interval) for the diagnosis of late onset Alzheimer's disease (LOAD), vascular dementia (VAD) or mild cognitive impairment (MCI) in subjects with low levels (below the median value=104 KU/L) of serum arylesterase. Covariates: age, gender, hypertension, CVD, diabetes, smoking status, and HDL-C.

Discussion

Recent works by ourselves [14–16] and others [17, 35] suggest that in dementia OxS might be not only a limited phenomenon occurring within the brain, but might also be present at systemic level. Indeed, in these large studies, LOAD, VAD, as well as MCI patients showed significantly higher concentration of serum OxS markers compared to cognitive healthy individuals [14, 15, 17]. It is tempting to speculate that, given the thriving sources of ROS in the brain (Aβ aggregates, microfibrillary tangles, neuroinflammation etc.) [12, 13] the in situ formed byproducts of biomolecular damage might diffuse to systemic circulation. However, in contrast with this speculation, F(2)-Isoprostanes (reliable markers of lipid peroxidation [36]) were found to be elevated in the brain [37], but not in the peripheral body fluids of LOAD and MCI patients compared to controls [38]. Thus, there might be systemic rather than local factors with either pro- (e.g., low grade chronic inflammation associated with dementia [2, 3]) or anti-oxidant function, which may account for the observed alteration of circulating OxS markers.

The prevention and protection against insult by free radicals can be afforded by low molecular antioxidants (especially vitamin C, E, uric acid, etc.) and some enzymes [10, 36]. Notably, compared to the non-enzymatic antioxidants, the biological catalysts are, by definition, faster and more efficient, even at low concentration, in scavenging reactive species [39]. There is a corollary of evidence demonstrating that paraoxonase and arylesterase activity, elicited by PON-1, and ferroxidase activity of Cp (and other still obscure molecules) exert a potent protective role in vivo against OxS-induced damage [20–25].

In the present study, we devoted our attention on the relationship of these enzymatic antioxidant activities and dementia in a large sample of older individuals including 223 MCI, 65 VAD, 162 LOAD patients, as well as 143 cognitively healthy controls. We found that, while paraoxonase, FeOx I and II activities were unmodified, arylesterase status was significantly and similarly reduced in the three groups of patients compared to controls. Moreover, the probability of being affected by MCI, LOAD, and VAD was higher in subjects with low serum levels of this PON-1

activity, independent of potential confounding factors, such as age, smoking, CVD, hypertension, diabetes and HDL-C. Relevant to this regard, the analysis of possible associations between the comorbidities considered in the study and arylesterase revealed that only hypertension was related to a significant decrease in this enzymatic activity (Wilcoxon-Mann-Whitney: p=0.02; Spearman correlation analysis: r=−0.097, p=0.02; Supplemental Material: Tables 5 and 6). This, although weak, association was also found by others [40–42] and can be due to the, widely documented [43], pivotal role of OxS in the genesis of hypertension.

The discovery of the physiological features of PON-1, as well as that of single nucleotide polymorphisms (Q192R and L55M are the most investigated variants in the coding sequence) able to affect its activity, raised the interest of researchers about the possible role of this serum enzyme in the development of VAD and LOAD [44–46]. In agreement with previous findings [44–46], we did not find any association between the susceptibility for LOAD, VAD or MCI and Q192R phenotypes, determined by the well established dual-substrate method [34]. On the one hand, these 'disappointing' results may be ascribed, as stated by Erlich [46] et al. and Camps et al. [47], to the multiple polymorphisms in PON-1 gene that can influence the catalytic function of the enzyme; on the other hand, it might depend from the complex effect of genetic variants on Alzheimer progression [48]. In contrast with the large amount of data on PON-1 genetic variants, only a relatively few studies have examined the relationship between biochemical determinants of this HDL-associated protein and dementia [29, 30, 35, 49]. Although showing an overall consistency with our results, currently available studies on this topic are, in our view, affected by some important limitations, in particular small sample size [35] and lack of control over relevant potential confounding factors (i.e., comorbidities [30, 49] and HDL concentration [29]). The latter limitation could also explain the absence of significant difference in arylesterase activity between MCI and controls revealed by the study of Wehr at al. [30]. It is important to notice as this work, together with the work by Dantoine et al. [29] (who measured only paraoxonase activity of PON-1), are the only published studies dealing with the pre-clinical stage of dementia.

In our opinion, the inclusion of a large number of MCI patients is an important strength of our work, since this allowed us to demonstrate that the alteration of serum arylesterase levels might be an early event in the development of dementia. This early decrease in antioxidant protection by PON-1 might be one of the reasons for the exacerbation of OxS observed in blood of patients experiencing this prodromal phase of dementia. It is well recognized that the anti-atherogenic properties of PON-1 are characterized by its ability to hydrolyze lipid peroxides in human atherosclerotic lesions [18, 20, 47]. This enzymatic action has also a systemic impact, since PON-1 seem to strongly contribute to lowering the plasma concentration of structurally distinct fatty acid oxidation products [20].

Unlike PON-1, there are still no robust epidemiological data in support of the systemic antioxidant effect of Cp and other serum enzyme (with still unknown function and structure) with iron oxidase ability. These observations are in apparent contradiction with the widely recognized relevance of Cp, the most potent FeOx enzyme, in getting rid of Fe^{2+} [24, 25], the main trigger of hydroxyl radical burst in general circulation [10, 23]. However, it has to be considered that Cp concentration, and related activity, may change in relation to several physiological and pathological conditions (e.g., copper deficiency, hepatic diseases and intoxications, etc.) [24, 25, 50, 51]. Besides, emerging in vitro evidence have demonstrated that Cp may enhance the oxidation of DNA [50] and LDL, both by intact Cp [51] and after release of redox-active copper by low pH [52]. The aforementioned factors influencing the antioxidant properties of Cp may account for the lack of association regarding this marker, and for the discrepancies with the other few small studies on this topic [26–28].

The main limitation of the present investigation is represented by its cross-sectional design, which precludes our ability to establish any temporal relationship between the peripheral markers examined and the occurrence of MCI or dementia. A longitudinal study on older cognitive healthy or MCI subjects, with sequential measurements of arylesterase at different time points, could help to address this still unsolved issue. Moreover, a 'residual confounding' bias might be present for some covariates (e.g., hypertension, diabetes, CVD) included into multivariate models as dichotomous (dummy) variables, and this should be considered as another potential limitation of the study.

Finally, our data do not allow us to ascertain whether the association between low arylesterase activity and the risk of having MCI or dementia might be explained by the decrease in the defense against OxS or by other factors. Indeed, PON-1 is an enzyme with multiple physiological functions and roles. Relevant to this notion, studies conducted in PON-1 knockout mice have shown that PON-1 may serve as a key determinant in the detoxification of organophosphate pesticides that, in turn, are regarded as strong risk factors for neurological diseases, such as LOAD and Parkinson's disease [53].

Conclusions

In a large sample of older individuals, we found that the likelihood of being affected by MCI, LOAD, or VAD was significantly, and similarly, increased in subjects with low level of serum arylesterase activity of PON-1, independently of possible confounders. Thus, taken together our data are consistent with a possible involvement of PON-1 in the early stages of dementia pathogenesis. However, longitudinal studies are warranted in order to establish a cause-effect relationship between reduced arylesterase activity and dementia.

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