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Original Article

Dyslipidemia, altered erythrocyte fatty acids and selenium are associated with dementia in elderly Nigerians

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ABSTRACT: Dyslipidemia, reduced omega-3 and -6 fatty acids and antioxidative nutrients are modulatory risk factors associated with dementia. Diet, genetics and environment interact with nutritional metabolism and susceptibility to neurodegeneration. This study investigated the relationship between erythrocyte fatty acids and selected antioxidant nutrients in elderly Nigerians with vascular dementia (VD) and Alzheimer's disease (AD). Forty VD (69.03±8.19 years) twenty AD (71.06±5.0 years) and forty control (67.5±6.8 years) subjects were studied. Anthropometric indices, blood pressure (BP) and body mass index (BMI) were measured in all subjects. Venous blood sample was drawn from all subjects and erythrocytes separated for the determination of fatty acids. Plasma lipids, selenium and vitamin E levels were also measured. There were no differences in BMI, weight and height among the three groups except for systolic BP that was lower in VD (148.3±41.8mmHg) than AD (156±36mmHg). Docosahexanoic acid and eicosapentanoic acid were lower in VD (6.3±2.2 and 2.0±1.6% total fatty acids [TFA]) and AD (5.4±3.1 and 3.0±1.7 %TFA) respectively than in controls (8.9±3.8 and 6.0±4.7%TFA). No variation was recorded in linolenic and arachidonic acids. Significant increases in triglycerides, LDL-cholesterol and decreased HDL-cholesterol were observed in both VD and AD when compared to controls ($p<0.05$ in all cases). Plasma selenium levels were significantly decreased in VD and AD than in controls. Eicosapentanoic and linolenic acids concentrations were negatively correlated with plasma total cholesterol. Low levels of erythrocyte omega-3 fatty acids and plasma selenium concentrations are associated with occurrence of vascular dementia and Alzheimer's disease in elderly Nigerians.

KEYWORDS: Antioxidants; Docosahexanoic acid; Eicosapentanoic acid; Lipoproteins

INTRODUCTION

Low dietary intake and plasma concentration of omega-3 polyunsaturated fatty acids (PUFA) are associated with cognitive decline and dementia risk[1]. The etiology of sporadic Alzheimer's disease (AD) is multifactorial. In Yorubas of African descent, it was observed that age, genetic variations, cultural, lifestyle and environmental factors like habitual diet may play important roles in the neurological disorder [2, 3]. It was hypothesized that very long chain omega-3 PUFA as present in fish oil could be responsible for interindividual variation in the rate of decline cognitive performance over time in the general elderly population[4]. Kalmijn et al.[5] reported that increased docosahexanoic acid (DHA) intake was protective of cognitive decline and that low concentration of DHA was found in brain tissue of persons with AD.

Brain cholesterol alters the degradation of amyloid precursor protein, which contributes to the pathogenesis of AD. Some workers suggested that reduced cellular cholesterol levels promote tau phosphorylation in neurons, inhibit dendrite outgrowth and synaptogenesis, and induce neurodegeneration[6]. The gene coding for the low-density lipoprotein receptor-related protein gene, which is the ApoE receptor and resides on chromosome 12, may be associated with the expression of late-onset AD[7].

Lipoproteins are targets for oxidation. It was reported that cholesterol oxidation in the brain is of relevance in pathogenesis of vascular dementia (VD) cases with mixed pathology [8]. Long chain PUFAs in brain include both omega-3 (e.g DHA, 22:6n-3) and omega-6 (e.g arachidonic acid (AA), 20:6n-6) PUFAs. They are prone to free radical attack and hydrogen abstraction leading to oxidative damage [9]. Oxidative damage in the form of lipid peroxidation is known to play a significant role in pathogenesis of neurodegeneration in AD [10]. Laboratory studies show that markers of oxidative damage are elevated in early AD[11] and this is associated with lower plasma antioxidant levels[11].

When there is a weakening of antioxidant defence or excessive production of free radicals, a state of oxidative stress occurs, if uncontrolled, these free radicals damage different biological targets

such as lipids [12]. Lipophilic antioxidants such as vitamin E is neuroprotective, essential for normal brain function, accumulates in the brain and decrease peroxidation of brain lipids in animal models [13,14]. Evidence suggests that decreased levels of antioxidant vitamins lead to higher susceptibility to oxidative stress and a higher grade of low–density lipoprotein (LDL) cholesterol oxidation as found in VD patients[6]. Epidemiological studies support antioxidant intake as a means of reducing AD risk from oxidative damage.[15, 16]

In humans, selenium deficiency has been implicated in etiology of cardiovascular disease and conditions in which oxidative stress and inflammation are prominent features [17]. Selenium deficiency also interferes with normal conversion of alpha-linolenic acid (ALA) to eicosapentanoic acid (EPA) and DHA leading to increased omega 6: omega 3 PUFA ratios [18]. Glutathione peroxidase (a selenium containing enzyme) and vitamin E inactivates the damaging effects of intermediates of lipofuscin metabolism [19]. Lipofuscin is formed by reactions of hydrogen peroxide with PUFA resulting in formation of organic free radicals which could damage neuronal DNA. This may have a direct toxic effect on brain cells [19]. This study is aimed at studying the relationship between omega-3, -6 fatty acids and selected antioxidant nutrients in elderly Nigerians with vascular dementia (VD) and Alzheimer's disease in comparison to controls.

MATERIALS AND METHODS

Study population

One hundred subjects (60 males, 40 females; mean age: 65.6±6.71 years) were enrolled in this study. These comprised of:

- Forty patients (24 males, 16 females; 69.03±8.19 years) attending the Neurology clinic at the Medical Outpatient Unit of the University College Hospital, Ibadan were diagnosed as suffering from vascular dementia using the National Institute of Neurological Disorders and Stroke (NINDS) criteria which includes incidence of cerebrovascular disease with or without history of stroke, onset of dementia within 3 months following a recognized stroke and abrupt or fluctuating deterioration in cognitive functions [20]. Diagnosis by the Consultant Neurologist also included taking a medical history, performing a physical and neurological examination, and administering a neuropsychological battery using Mini-Mental State Examination (MMSE).

- Twenty patients (9 males, 11 females: mean age: 71.06 ±5.0 years) suffering from Alzheimer's disease were diagnosed using the National Institute of Neurological and Cognitive Disorders (NINCD) and Stroke/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria. These include: dementia established by examination and objective testing, deficits in 2 or more cognitive areas, progressive worsening of memory and other cognitive functions, onset between ages 40 and 90 years and establishment of absence of systemic disorders or other brain diseases [21].

- Forty apparently healthy subjects (27 males, 13 females; mean age: 67.5 ± 6.83 years) who had no report of endocrine diseases, diabetes mellitus, hyperlipidemia or hypertension and had not used any medications or drugs influencing fat metabolism during the last 3 months before commencement of the study were selected as controls.

Each participant underwent an interview with the aid of a structured questionnaire of general health and detailed dietary habits using a food frequency questionnaire followed by a standard assessment of alcohol and fish consumption during the preceding 3 months. All the subjects gave their informed consent prior to participation in the study. The study was approved by the University of Ibadan / University College Hospital Ethical Review Committee. **Inclusion criteria:** Patients with VD and AD and that are over 60 years old. **Exclusion criteria:** Individuals that use psychotropic drugs or consume alcohol on a regular basis, and also individuals below 60 years old; Unwillingness to participate in the study.

Blood sample collection

Blood sample (10 ml) was drawn by venipuncture after 10-14 hours fast and was carefully dispensed into ethylenediaminetetraacetic acid (EDTA)-containing bottles. Samples were immediately placed on ice before centrifugation. Whole blood was centrifuged at 4^{O}C for 10 min at 2500rpm; the plasma was immediately separated from erythrocytes, and then freshly frozen under nitrogen at -80^{0}C, not later than 30 min after puncture.The erythrocytes were washed four times at 4^{O}C and centrifuged at 2500rpm for 10min. The plasma and buffy coat were removed after centrifugation. This procedure was done twice, leaving a substantially hemoglobin-free pellet of erythrocyte membranes, which was resuspended in twice its volume of phosphate buffer saline and stored at -70^{O}C until analyzed for fatty acids. The separated plasma sample was used for α-tocopherol, selenium and lipid analysis.

Plasma lipids and fatty acid analysis

Plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) cholesterol were quantified by commercially available enzymatic kits (Boehringer Mannheim, Mannheim, Germany). LDL- cholesterol was calculated using the Friedewald formular (LDL-C: TC - TG/5 - HDL-C). Erythrocyte fatty acids were determined using the method of Pandey et al [44] . Fatty acid methyl esters (FAMEs) were prepared by methylating free fatty acids with methanol sulphuric acid. High Performance Liquid Chromatography was carried out using Waters 616/626 LC chromatograph equipped with Waters 501 pump solvent programmer and Waters UV detector. Absorbance was measured @ 300nm. A 90cm x 0.64cm: bondapak C18 column was used with acetonitrite (8:20v/v) as eluent at a flow rate of 0.50ml/min. FAMEs were identified by comparison with internal standard (C17:0). Peak retention times for total fatty acids (TFA) were identified by injecting known standards. FAMEs from C12:0 through C22:6 were expressed as percent TFA. Precision of fatty acids measurement was determined by repeat analysis of a pooled plasma sample; 1 pooled plasma sample was assayed for every 20 samples.

Measurement of α-tocopherol and selenium

α-Tocopherol was determined as a major form of vitamin E in plasma and was quantified by reversed-phase high-performance

liquid chromatography (HPLC) with electrochemical detection as previously described [22]. Plasma selenium was estimated using atomic absorption spectrophotometry.

Statistical analysis

Lipid levels, erythrocyte fatty acids content and other potential risk factors were compared among VD, AD and non-demented subjects using SPSS version 14.0. Data are reported as mean ± standard deviation (SD). Means were compared by analysis of variance; Chi-square test was used for categorical data while the relationships between variables were determined using Pearson correlation coefficient. Statistical significance was defined as $p < 0.05$.

Other measurements

Anthropometric indices were measured which included weight in light clothing with shoes off using analogue bathroom scale, and height using standiometre. Body mass index (BMI) was computed as weight (kg)/height (m^2). Subjects were considered as smokers (current or past) at the time of blood sampling. Percentage alcohol intake was considered as ≥ 2 drinks per day (1 drink was defined as 13ml alcohol). High blood pressure was defined as systolic blood pressure (BP) ≥140mmHg or a diastolic BP ≥ 80mmHg [23]. Subjects with high BP by this definition or those taking antihypertensive medication such as β-blockers, calcium antagonists or diuretics were considered hypertensive [24].

RESULTS

General characteristics of patients and controls are displayed in Table 1. No significant differences in age, BMI and diastolic blood pressure were seen in VD and AD patients but increase in systolic BP was significant in AD compared with VD patients. The percentage consumption of fish in VD and AD was similar and higher than controls. Approximately 62.5% of VD and 25% of AD patients were hypertensive.

TABLE 1 General characteristic of VD, AD and control subjects

Parameters	VD (n=40)	AD (n=20)	Control (n=40)
BMI(kg/m^2)	26.4±3.4	26.1±2.6	25.9±2.7
Smoking (Y/N)	9/31	2/18	1/38
Hypertension (Y/N)	25/15	5/15	0/40
Alcohol intake (%)	23	22	18
Fish intake (%)*	20	20	25
SBP(mmHg)	148.3±41.8	156±36.0	141±24.2†
DBP(mmHg)	85.3±20.9	89±18.0	81±15.1
MMSE Score	12.3±2.2	12.0±3.4	26.1±2.2§

*1mg/d = 1.05 fish servings/week [25]; † $p<0.05$ vs VD and AD; § $p<0.05$ vs VD and AD. SBP – systolic blood pressure; DBP – diastolic blood pressure

From Table 2, differences in mean plasma TG, LDL-C and HDL-C in all patients were significant compared to control. In contrast, there was no significant change in the mean plasma total cholesterol in the patients and control group. In VD patients, mean values of TG and LDL-C were significantly higher and HDL-C lower ($p<0.05$ in all cases) than in AD. The mean percentage contributions of n-6 PUFAs: AA and linolenic acid (LA) showed minimal variations between the dementia subtypes and control subjects. Mean contributions of DHA and EPA were significantly lower in the patients than the values in controls. When patients with VD and AD as well as control subjects were compared according to their mean values of plasma vitamin E and selenium, plasma selenium concentration was higher in patients compared to controls but changes in vitamin E among the different groups were not significant (Table 3).

TABLE 2 Plasma lipids and erythrocyte fatty acids contents in VD, AD and control subjects

	VD (n=40)	AD (n=20)	Control (n=40)
TC (mmol/l)	4.3±28.4	4.0±27.2	3.54±26.2
TG (mmol/l)	3.24±41.9	2.85±36.1	1.80± 26.3*
HDL-C (mmol/l)	1.03 ± 20.4	1.40 ±14.8	1.60 ± 14.9**
LDL-C (mmol/l)	2.80±32.4	2.21 ±20.2	1.58 ± 21.1**
TFA (mmol/l)	37±2.9	35.1±3.1	39.2±2.7
AA (% TFA)	13.6±2.4	14.0±2.3	14.3 ±3.5
LA (% TFA)	10.1±2.5	10.3±2.4	8.1 ± 3.3
DHA (% TFA)	6.3±2.2	5.4±3.1	8.9 ± 3.8#
EPA (% TFA)	2.0±1.6	3.0±1.7	6.0 ± 4.7#

* $p<0.05$ vs VD and AD, ** $p=0.000$ vs VD and AD, # $p=0.002$ vs VD and AD.

In order to ascertain possible relationships among the biochemical parameters analyzed in both dementia subtypes with the exclusion of control subjects, Pearson correlation coefficient showed that in VD patients, consistent positive correlations were present between triglyceride and total cholesterol, LDL cholesterol and total cholesterol and LDL cholesterol and triglyceride. On the other hand, there were negative correlations between EPA and total cholesterol and LA and total cholesterol (Table 4). In AD patients, there were no significant correlations among the parameters analyzed (data not shown).

DISCUSSION

The primary purpose of the present study was to identify possible relations between the expression of specific putative atherogenic risk modulating factors, antioxidative nutrients and the incidence of dementia in this community. In the present study the patients were a mixed population of elderly male and female adults suffering from either vascular dementia or Alzheimer's disease. Lifestyle factors including habitual diet, cigarette smoking and alcohol intake are believed to be major contributors to the development of VD and AD. The findings in this study showed that a larger percentage of VD (29%) and AD (11.1%) patients were either current smokers or had smoked before when compared with

control subjects (2.6%). In a review of risk factors and post stroke dementia by Pasquier et al [26], it was postulated that cigarette smoking was a common risk factor for both Alzheimer's and vascular disease.

TABLE 3 Plasma selenium and vitamin E levels in all subjects

	VD (n=40)	AD (n=20)	Control (n=40)
Selenium (µmol/l)	1.39±0.7*	1.48±0.57*	2.06±0.53
Vitamin E (µmol/l)	1.63±0.74	1.73±0.86	1.78±0.68

*significantly different from controls

This study showed that increased triglyceride, LDL-cholesterol and decreased HDL-cholesterol concentrations in plasma were associated with incidence of VD. In addition, total cholesterol concentration appeared to be elevated in VD and AD patients compared with control subjects. These findings also suggest that hypertriglyceridemia and elevated LDL- cholesterol may be among the intermediary mechanisms linking hyperlipidemia to VD. This observation is in agreement with some studies [8,27] and at variance with others [28]. A previous study suggested that elevated serum lipids may enhance atherogenesis of the extracranial and intracranial arteries causing an increase in blood viscosity which in turn decreases cerebral perfusion and impair cognitive performance [29].

To operationalize the diagnosis of dementia for this study, we developed a composite variable with a positive diagnosis when both a clinical diagnosis of dementia or one of its types were documented and the patient had a MMSE score less than 24. This cutoff has been shown to improve diagnostic accuracy [30]. Covariates included age, gender, smoking, alcohol intake, blood pressure, medical diagnosis (stroke, hypertension and depression), family history of dementia or AD, cholesterol level, LDL-cholesterol, HDL-cholesterol and triglyceride. Both of the dementia groups were similar in BMI, alcohol intake, fish consumption and MMSE score. A limitation of this study is the accuracy of the diagnosis of dementia. However, the high rate of concordance between clinical diagnosis and cognitive testing result suggest accuracy of dementia diagnosis.

The systolic blood pressure was significantly higher in AD and VD patients than in controls while the change in diastolic blood pressure was not significant. It is noteworthy that the blood pressure values for AD and VD patients (156/89 and 148/85 respectively) were higher than the cut-off value of 140/85mmHg for diagnosis of hypertension [23]. In their study on association of cognitive function with high blood pressure, hypertension and high pulse rate in persons aged 60 years and older, Obisesan et al [31] reported that optimal blood pressure (120/80mmHg) was associated with best cognitive performance and that at age 70 years and older, high blood pressure, hypertension and uncontrolled blood pressure were independently associated with poorer cognitive function than normal blood pressure. It is therefore suggested that optimal control of blood pressure may be useful in preventing neurocognitive loss in the aging population. The causal role of vascular risk factors in different types of dementia has been linked to sclerosis of small cerebral arteries and arterioles which is considered to be responsible for diffuse periventricular white matter abnormalities, which play an important role in the development of VD [6]. It was hypothesized that high concentrations of LDL-cholesterol and low levels of HDL-cholesterol are independent risk factors for coronary heart disease and carotid artery atherosclerosis [32]; this in turn may lead to cognitive impairment through cerebral hypoperfusion or embolism [33]. HDL-cholesterol particles have also been reported to play a role in the removal of excess cholesterol from the brain by interaction with ApoE and heparan sulfate proteoglycans in the subendothelial space of cerebral microvessels and this mechanism has been linked to low incidence of small-vessel disease [34].

TABLE 4 Correlation coefficient matrix of parameters in VD and AD

Variables	TC	TG	LDL-C	EPA	LA
TC	NS	0.395*	0.333*	-0.353*	-0.333*
LDL-C	NS	0.458**	NS	NS	NS
EPA	-0.353*	NS	NS	NS	NS
TG	0.395*	NS	0.458	NS	NS
LA	-0.333*	NS	NS	NS	NS

*significant @ p<0.05 level, **p<0.01; NS – not significant

There is increasing scientific interest in the hypothesis that very-long-chain n-3 PUFAs, as present in fish or fish oil and supplements rich in n-3 fatty acids are beneficial for the maintenance of cognitive performance in adults. This hypothesis is corroborated by the pattern of erythrocyte fatty acid changes in this study which shows that the percentage of DHA and EPA, were significantly decreased in VD and AD patients compared to control subjects. Several observational studies, conducted among older adults reported that participants in those studies with high erythrocyte levels of eicosapentanoic acid and docosahexanoic acid - which are the most abundant n-3 PUFAs in erythrocyte - had a lower risk of experiencing cognitive decline [24], [35]. However others have reported no such associations [15, 36]. Some of these studies also evaluated the association between fish consumption and cognitive performance and reported a lower incidence of AD [37] or a trend toward a lower risk of cognitive decline [38] with increasing fish intake. In the present study the average percentage fish intake in patients with the dementia subtypes was slightly higher than the control subjects. It could be possible that these control subjects represent a population of people with relatively unhealthy dietary habits, such as inadequate consumption of diets rich in n-3 PUFAs.

There were no significant differences in erythrocyte levels of DHA and EPA in vascular dementia as compared to those in Alzheimer's disease patients probably suggesting a possible relationship between n-3 PUFAs and low cognitive function irrespective of the subtypes of dementia. This is more likely as both VD and AD patients studied scored less than 24 points on the

MMSE scale. Dietary n-3 PUFAs had been shown to improve brain functioning in animal studies and it was hypothesized that dietary intake of n-3 fatty acids and weekly consumption of fish may reduce the risk of incident Alzheimer's disease [37]. Nevertheless, the findings in this present study are in line with earlier report by Heude et al [24] which showed that lower proportions of n-3 PUFAs in erythrocytes are associated with a higher risk of cognitive decline. Hence, there is the possibility that a more detailed cognitive assessment in a larger population may show significant associations in fish consumption and cognitive impairment in this community.

A suggested mechanism for the cardio-protective effect of n-3 fatty acids focused on their influence on eicosanoid metabolism, inflammation, beta oxidation, endothelial dysfunction, cytokine growth factors, and gene expression of adhesion molecules [39]. Cerebrovascular disease might play a role in vascular dementia [34,6]. N-3 fatty acids are also neuroprotective by suppressing the synthesis and release of interleukins and TNF-alpha (which is neurotoxic) and modulation of hypothalamic-pituitary-adrenal anti-inflammatory responses in the nervous system [39].This shows a close association between the central nervous system and dietary n-3 fatty acids. High concentrations of DHA and EPA are associated with cardiovascular benefit. Trials showed reductions in cardiovascular events of 19-45% in subjects receiving n-3 fatty acid supplementation containing EPA and DHA. Patients with hypertriglyceridemia can also benefit from treatment with 3-4g daily intake of DHA and EPA [40]. Previous literature from this community emphasized or studied the incidence and risk factors of VD and AD. With this study, erythrocyte PUFAs, total plasma lipid profile, selenium and vitamin E were all determined in the two groups of neurodegenerative disorders. Low level of plasma selenium was a constant feature in VD and AD patients but not in control subjects.

Although the slight decrease in plasma values of vitamin E observed in patients in comparison with controls in this study was not significantly different, low levels of this vitamin may still undoubtedly play an important role in the occurrence of these neurodegenerative disorders. Several studies found that low intake and reduced serum vitamin E was associated with higher risks of AD and vascular dementia [41,42] and that Alzheimer' disease patients on vitamin E supplement survived longer than those not getting supplements [43]. It could be deduced from results obtained in this study that vitamin E is unlikely to be responsible for the severity and progression of VD and AD among Nigerian Africans when compared with the white populations of the developed countries [41,42].

Results from this case-control study showed that low levels of omega-3 fatty acids and selenium were strongly associated with the occurrence of dementia in the elderly Nigerian population among whom there is lack of awareness on the benefits of adequate consumption of fatty cold water fish that are rich sources of eicosapentanoic and docosahexanoic acids known to alleviate neurodegeneration. A prospective study investigating the effect of omega-3 fatty acids and antioxidant vitamins on pathogenesis of dementia in the elderly would shed more light on the observed associations.

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