

DECREASED SERUM LIPIDS IN PATIENTS WITH PROBABLE ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial disease but its aetiology and pathophysiology are still not fully understood. Epidemiologic studies examining the association between lipids and dementia have reported conflicting results. High total cholesterol has been associated with both an increased, and decreased, risk of AD and/or vascular dementia (VAD), whereas other studies found no association. The aim of this study was to investigate the serum lipids concentration in patients with probable AD, as well as possible correlation between serum lipids concentrations and cognitive impairment.

Our cross-sectional study included 30 patients with probable AD and 30 age and sex matched control subjects. The probable AD was clinically diagnosed by NINCDS-ADRDA criteria. Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were determined at the initial assessment using standard enzymatic colorimetric techniques. Low-density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) levels were calculated. Subjects with probable AD had significantly lower serum TG ($p < 0,01$), TC ($p < 0,05$), LDL-C ($p < 0,05$) and VLDL-C ($p < 0,01$) compared to the control group. We did not observe significant difference in HDL-C level between patients with probable AD and control subjects. Negative, although not significant correlation between TG, TC and VLDL-C and MMSE in patients with AD was observed. In the control group of subjects there was a negative correlation between TC and MMSE but it was not statistically significant ($r = -0,28$). Further studies are required to explore the possibility for serum lipids to serve as diagnostic and therapeutic markers of AD.

KEY WORDS: Alzheimer's disease, lipids, cognitive impairment, Mini-Mental State Examination.

INTRODUCTION

Dementia is a clinicopathological state whose name literally means "loss of the ability to think." Alzheimer's disease (AD) and vascular dementia (VAD) are the most common forms of dementias. Alzheimer's disease is a multifactorial disease but its aetiology and pathophysiology are still not fully understood. Studies so far have shown that pathomechanisms responsible for AD are excessive production of nitric oxide (NO), hypoperfusion of brain vasculature and increased oxidative stress. Oxidative modification of lipids in serum and cerebrospinal fluid have important role in AD pathogenesis (1). Efforts have been made to discover biological markers for different types of dementia during the last few decades. Such markers may play a key role in early diagnosis and management of these disorders. Serum lipids are measured routinely in everyday practice and, therefore may constitute tempting potential biomarkers for many diseases (2). Epidemiologic studies examining the association between serum cholesterol and dementia have reported conflicting results. Among longitudinal studies, high total cholesterol (TC) level has been associated with both an increased and decreased risk of AD and/or VAD, whereas other studies found no association (3). In a Swedish study, low cholesterol level was associated with dementia even 9 or more years before the diagnosis (3). In another study, although high levels of cholesterol at midlife represented a risk factor for AD, there was no detectable difference in cholesterol levels in late life (4). At least two autopsy studies supported that midlife, but not late life lipids are somehow related to Alzheimer's disease-type pathology (5,6). In a previous report from the Honolulu-Asia Aging Study, low midlife TC level was associated with a lower number of neuritic, amyloid plaques and neurofibrillary tangles (the characteristic pathologic changes of AD), while late-life TC and low density lipoprotein cholesterol (LDL-C) levels were not associated with any of the AD neuropathologic markers (5). In cross-sectional studies, lower HDL-C levels have been associated with lower Mini-Mental State Examination (MMSE) scores (7) and with higher risks for dementia (8, 7, 9) and AD (10). However, most longitudinal studies have failed to detect similar associations: HDL-C levels have not been associated with AD (11–15, 16), VAD (11, 13, 15), or other forms of dementia (15).

The aim of the study

The aim of this study was to investigate serum lipids concentration in patients with probable AD,

as well as possible correlation between serum lipids concentration and cognitive impairment tested by MMSE in patients with probable AD.

MATERIALS AND METHODS

Patients

The study was designed as a cross-sectional study which included patients with probable AD and control subjects. Group of patients with probable AD (AD group) enrolled in the present study comprised of 30 patients, 24 females and 6 males, with clinically diagnosed probable AD. Control group (CG) included 30 community dwelling age-matched apparently healthy, asymptomatic persons without dementia (22 females and 8 males). All subjects were aged 65 and over. Patients in AD group were patients currently institutionalized at specialized unit for patients with dementia within Health-Care Hospice for person with disabilities and other persons in Sarajevo, Bosnia and Herzegovina. The probable AD was clinically diagnosed by standardized clinical examination conducted by a senior staff neurologist and psychiatrist by NINCDS-ADRDA criteria (17). Subjects underwent history and clinical examination. Anamnesis was taken from patient's caregivers by a one-to-one interview with special emphasis on previous symptomatic cerebrovascular diseases. Clinical examination included physical examination, risk factors assessment for probable Alzheimer disease and Cranial Computed tomography (CT). Physical examination included clinical neurological examination, blood pressure and Body Mass Index (BMI) measurement. BMI for each subject was calculated (weight in kilograms divided by height in meters squared). Height was measured with stadiometer and weight was measured with Toledo self-zeroing electronic digital scale (Mettler-Toledo, Inc., Worthington, OH.). Trained persons measured blood pressure using a mercury sphygmomanometer (MD10XX, MEDI, Shanghai, China) on the right arm after at least a 5-min rest. For both groups of subjects, exclusion criteria were positive history of cardiovascular or thyroid disease, chronic inflammatory disease (asthma and rheumatoid arthritis), hepatic or renal insufficiency, cancer and VAD. Exclusion criteria for AD patients was AD associated with cerebrovascular lesions or probable VAD (so called "mixed dementia") assessed with Hachinski ischemic score (HIS). Approval for the study was obtained by the local ethics committee. All procedures on human subjects were performed in the accordance with

Helsinki Declaration of 1975. Informed consent was obtained from subjects and caregivers upon careful explanation of the study procedure.

Clinical diagnosis of Alzheimer's disease

The probable AD was clinically diagnosed by standardized clinical examination conducted by a senior staff neurologist and psychiatrist by NINCDS-ADRDA criteria and MMSE score. NINCDS-ADRDA criteria for clinical diagnosis of probable AD (i.e., insidious onset of cognitive decline with progressive deterioration and the exclusion of all other causes of dementia by history, physical examination, and laboratory tests) was based on National Institute of Neurological and Cognitive Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria (17).

Global cognitive function was tested with the 30-point MMSE score (18). This screening test was originally created for a clinical setting (18) and is used extensively in epidemiologic studies (19). The test was administered by specially trained research assistants. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction. If fewer than four individual items (out of 20) were not answered by the subject, these were rated as errors (20). If a subject did not answer four or more individual items, the total MMSE score was considered missing. A score of less than 23 points on the MMSE indicated cognitive impairment (18). All patients had MMSE score ≤ 12 . All subjects in CG had a MMSE score of 26-30.

Hachinski ischemic score

Hachinski ischemic score is a test which helps differentiate patients with VAD from individuals with AD. The HIS assesses the presence of thirteen clinical features and attributes a total score of 18. The score above 7 suggests a diagnosis of multi-infarct dementia (MID) and if 4 or below, it suggests AD. This score has been validated in patients with pathologically confirmed MID and AD and allows for correct identification of MID and AD cases in 84% and 76% of cases, respectively (21). In our study all patients with probable AD had HIS 4 or bellow.

Cranial Computed tomography

Cranial CT was performed in 27 patients with probable AD, while remaining 3 were uncooperative and refused a CT scan. All CT scans were performed without con-

trast enhancement and with 8-mm continuous slices on spiral CT (SOMATOM Emotion Duo, Siemens, Erlangen, Germany) on Institute of Radiology of University of Sarajevo Clinics Centre. The scans were examined by a neuroradiology specialist. Analysis of CT scans showed that all patients with probable AD had cerebral atrophy and that 4 of these patients had also cerebellum atrophy.

Blood analysis

Blood samples for analysis were obtained from patients and subjects in fasting conditions from antecubital vein into siliconized tubes (BD Vacutainer Systems, PL6 7BP, Plymouth, UK.). Plasma TC, HDL-C and triglyceride (TG) levels were determined at the initial assessment using standard enzymatic colorimetric techniques, on automated apparatus (Dimension RxL Max, Dade Behring, Germany) at the Institute for Chemistry and Biochemistry University of Sarajevo Clinics Center. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald et al. formula (22). Very low density lipoprotein cholesterol (VLDL-C) levels were calculated by the formula: $VLDL-C = TG/2,2$.

Referral value using this method for serum total cholesterol level is < 5.2 mmol/L, triglycerides $< 1,7$ mmol/L, HDL-C $1.04 - 1,55$ mmol/L, LDL-C $1,4 - 4,5$ mmol/L and VLDL-C $0,13 - 0,90$ mmol/L. Hyperlipidaemia was defined as fasting serum triglycerides level $> 1,7$ mmol/L and cholesterol level $> 6,5$ mmol/L representing threshold for people older than 60 years without additional risk factors, or current treatment with lipid lowering therapy (23).

Statistical analysis

Statistical analyses were performed with Microsoft Office Excel 2003 and SPSS, version 12.0. Data are presented as mean \pm SEM. Data distribution was determined using the Kolmogorov-Smirnov test. Since data were normally distributed, statistical difference was tested with Student t-tests. Additionally, Pearson correlations were used as measures of association for the continuous variables. Statistical significance was set at $p < 0,05$.

RESULTS

The baseline characteristics of the two groups enrolled in the study are reported in Table 1. No difference emerged in age, systolic, diastolic blood pressure and BMI between AD and control group. Subjects in AD group had significantly lower MMSE score compared with the control group ($p < 0,0001$).

	AD group MMSE	Control group MMSE	p<
Age (year)	79,96 ± 0,95	77,53 ± 0,96	NS
SBP mmHg	133,23 ± 3,49	130,13 ± 3,12	NS
DBP mmHg	82,66 ± 1,58	82,30 ± 1,46	NS
BMI (kg/m ²)	23,71 ± 1,03	25,31 ± 0,54	NS
MMSE score	4,53 ± 0,62	28,33 ± 0,25	0,0001*

Data are presented as mean ± S.E.M. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: Body mass index; MMSE: Mini Mental State Examination; Alzheimer's disease (AD) group.

TABLE 1. Baseline characteristics of patients with probable Alzheimer's disease and control subjects

As presented in Table 2., negative, although not significant correlation between TG, TC and VLDL-C and MMSE in patients with AD was observed. In the control group of subjects there was a negative correlation between TC and MMSE but it was not statistically significant (r = -0,28). Subjects with probable AD had statistically significantly lower serum TG (p<0,01), TC (p<0,05), LDL-C (p<0,05) and VLDL-C (p<0,01) compared to control group (p<0,05). We did not observe statistically significant difference in HDL-C level between patients with probable AD and control subjects (Figure 1).

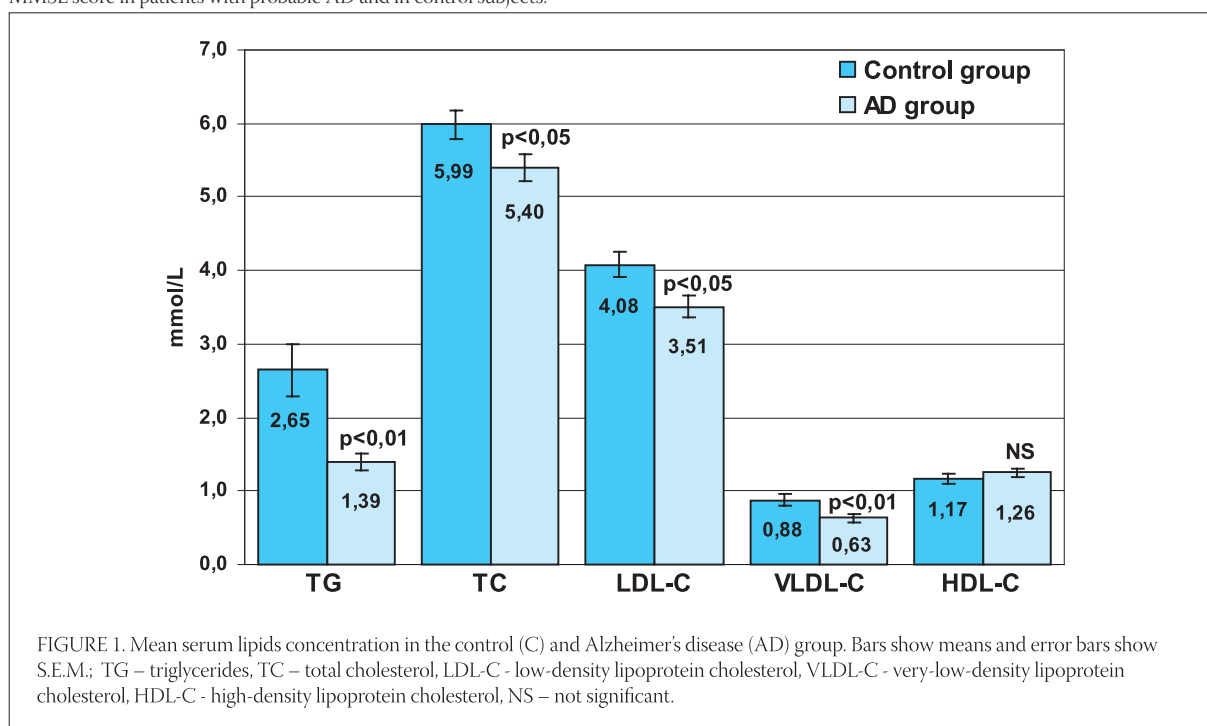
	AD group MMSE	Control group MMSE
TG (mmol/L)	r = - 0,25	r = 0,001
TC (mmol/L)	r = - 0,03	r = - 0,28
LDL-C (mmol/L)	r = 0,02	r = - 0,20
VLDL-C (mmol/L)	r = - 0,22	r = - 0,04
HDL-C (mmol/L)	r = 0,01	r = - 0,11

TG – triglycerides, TC – total cholesterol, LDL-C - low-density lipoprotein cholesterol, VLDL-C - very-low-density lipoprotein cholesterol, HDL-C - high-density lipoprotein cholesterol.

TABLE 2. Pearson correlation analysis between serum lipids and MMSE score in patients with probable AD and in control subjects.

DISCUSSION

In our cross-sectional study we found significantly lower serum lipids except of HDL-C in patients with probable AD compared to control subjects. However, measure of adiposity tested by BMI was not different in AD group compared to controls which suggests that decrease in serum lipids in patients might not be due to poor nutritional status. Several studies assessed the serum lipids in patients with AD, VAD and control subjects but the results are conflicting. A recent study, with approximately 5 years of follow-up, suggested that high cholesterol in late life was associated with a lower risk of AD (13). By contrast, two longitudinal studies (24,25) reported that high TC measured in mid-life was associated with an increased risk of dementia as long as 30 years later. Notkola et al. (24) found that high cholesterol was associated with a higher risk of dementia 30 years later, but before the onset of dementia cholesterol levels began to decline and as a result, low cholesterol was associated with dementia cross-sectionally. The authors noted that a decrease in cholesterol immediately before the dementia diagnosis was also predictive of dementia. Romas et al. (12) found that decreased TC level had significant inverse association with incident AD compared with nondemented elderly, independent of APOE genotype. In their study, no other lipoprotein fragment was associated with either prevalent or incident AD. Experimental studies suggest that high cholesterol accelerates the production of β-amyloid, the putative pathologic species in AD, by shifting amyloid precursor pro-



tein metabolism from alpha to beta cleavage products (26-28). However, cholesterol is an essential molecule for many physiologic processes and may have several beneficial effects as well. Cholesterol is a precursor of steroid hormones (estrogens, androgens, vitamin D), provides structural integrity and modulates fluidity of cell membranes, and is essential for basic synaptic integrity and neurotransmission. All these processes are compromised with aging and have been shown to be dysfunctional in patients with AD. In addition, *in vitro* studies have suggested that cholesterol acts as an antioxidant and therefore has a protective role in dementia pathogenesis, possibly through intercepting pro-oxidants to create oxysterols, which are less toxic than free radicals (3). However, it has been noted by Jacobs et al. (29) elsewhere that low (not high) cholesterol is associated with mortality among the elderly, and the authors suggested it is possible that cholesterol might play an important role in protecting against dementia. Therefore, we believe that an alternative explanations focusing on potentially neuroprotective properties of

cholesterol should be considered. It was speculated that high cholesterol may be protective through increasing gamma-glutamyltransferase. This enzyme plays a role in amino acid uptake and transport and could reduce the neurotoxic effects of amino acids (30). Hypertriglyceridemia has been confirmed as a cardiovascular risk factor. On the contrary, Dziedzic et al. (30) found that lower TG level is associated with more severe stroke but were unable to offer an explanation and potential biological mechanism responsible for inverse association between TG level and stroke severity. Dimopoulos et al. (2) found lower TG plasma levels in patients with dementia compared to the control group, but the difference was not significant. Several studies confirmed that the level of TG in the blood is not associated with memory and cognitive abilities (7,31). We did not find the data on the association between the lower triglycerides levels and risk for AD development in current literature. Unfortunately, we can not give an explanation for significantly decreased triglycerides levels in patients with possible AD.

CONCLUSION

Given that the determination of lipids in the blood is a routine, non invasive and cheap method we believe that monitoring of lipids in older individuals with dementia may be an additional tool in evaluation of patients with probable AD. Although further studies are needed serum lipids levels might serve as a potential biomarkers of AD severity.

List of Abbreviations

AD	-	Alzheimer`s disease
VAD	-	Vascular dementia
MID	-	Multi-infarct dementia
TC	-	Total cholesterol
TG	-	Triglyceride
LDL-C	-	Low-density lipoprotein cholesterol
HDL-C	-	High-density lipoprotein cholesterol
VLDL-C	-	Very low density lipoprotein cholesterol
NINCDS-ADRDA criteria	-	National Institute of Neurological and Cognitive Disorders and Stroke/Alzheimer`s Disease and Related Disorders Association criteria
MMSE	-	Mini Mental State Examination
NO	-	Nitric oxide
CT	-	Computed tomography

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