



GENDER ASSOCIATED LIPID AND APOLIPOPROTEIN PROFILE IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

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Abstract

The role of lipid parameters disorder in the development of age-related macular degeneration (AMD) is unclear. The aim of this study was to analyze lipid profile in these patients and to test the influence of gender on lipid profile of AMD patients, especially in the early and late form of the disease. 82 patients with AMD (mean age 70.3 yrs) and 80 age-matched control subjects were included in this study. Serum lipid and apolipoprotein levels were determined using standardized methods. AMD patients had significantly higher values of total cholesterol (P=0.000), HDL-cholesterol (P=0.0003) and LDL-cholesterol (P=0.000) compared to control group. Significantly higher values of apo A1 (P=0.039), apo E (P=0.002), total-cholesterol (P=0.000), LDL-chol. (P=0.026), total HDL-chol (P=0.000), HDL₃-chol. (P=0.005) and non-HDL-cholesterol (P= 0.029) were found in female AMD patients compared to males with AMD. Females with the advanced form of the disease had significantly higher total cholesterol (P=0.006), HDL-C (P=0.004), non HDL-C (P=0.05) and apo E (P=0.014) compared to males with the same form of the disease. There is a significant disorder of lipid parameters in AMD patients especially in females. More severe forms of AMD are followed by the increase of atherogenic lipoproteins and apolipoproteins, and females have higher values of these parameters compared to males with the same form of AMD.

INTRODUCTION

Age-related macular degeneration is the most common cause of visual impairment in the individuals over 50 years of age in developed countries. Although the definition of age-related macular degeneration differs in various studies, the condition is generally characterized by extensive drusen, often associated with pigmentary abnormalities (1). Drusen are visualized as yellow deposits under the retinal pigment epithelium and neurosensory retina. The clinical presentation of AMD includes drusen, hyperplasia of the retinal pigment epithelium (RPE), geographic atrophy, and choroidal new vessels (CNVs) (2).

The prevalence of early AMD (i.e. the presence of soft indistinct or reticular drusen or drusen with RPE degeneration or hyperpigmentation) is 18% in the population aged 65 to 74 years and 30% in the population older than 74 years (3).

Early pathological changes in age-related macular degeneration involve basal laminar deposits in Bruch's membrane (BrM), which comprises mostly wide-spaced collagen (4) and other materials, including laminin, membrane bound vesicles, and fibronectin. These materials could be seen even in the seventh decade of life in normal aging (5). Basal linear deposit, consisting primarily of granular and vesicular material with foci of wide-spaced collagen, appears in older persons and is more specific for AMD (6, 7). Molecules present in the Bruch membrane impart a negative electrostatic charge at physiologic pH. Age-related changes in glycosaminoglycans might alter this charge leading to changes of permeability properties of the Bruch membrane (8).

Aging is associated with biological changes in the eye, and may contribute to the pathogenesis of AMD, but they do not lead inevitably to AMD. Aging is also associated with cumulative oxidative injury (9). Advanced glycation end products accumulate in the Bruch membrane during aging (10). Specifically, there is increased lipidization, protein cross-linking, and protein deposition in the Bruch membrane with aging. Lipid accumulation in the Bruch membrane begins to increase substantially after the age of 40 (11). The rate of lipid accumulation under the macula may be higher than under the peripheral retina. Lipids seem to be derived from long-chain polyunsaturated fatty acids normally found in the outer segments. Changes in protein cross-linking, noncollagenous protein deposition and age-related lipid accumulation in the Bruch membrane may be the underlying cause of AMD (12).

It is believed that plasma lipoprotein concentration may be one of the risk factors for development of age-related macular degeneration in elderly patients. It is also considered that the concentration of apo A1, the major apolipoprotein in HDL, and apo B, the only apolipoprotein in LDL, could be better predictors of atherosclerosis than HDL and LDL cholesterol concentration (13). It has been documented that AMD is twice as prevalent in aging postmenopausal women compared to men of the same age. Individuals with $\epsilon 2$ genotype of APO E (c526C>T) had significantly 4.8 fold higher relative risk of AMD. These findings were found only in females who progressed with AMD suggesting that there may be gender-related factor implicated in pathogenesis of AMD.

The aim of this study was to analyze the concentration of serum lipids and lipoproteins (total cholesterol, HDL-, non-HDL- and LDL-cholesterol, triglycerides and HDL₂ and HDL₃-cholesterol subfraction) as well as apolipoproteins: apo A1, apo A2 apo B, apo E and Lp(a) in patients with age-related macular degeneration in order to explore the contribution of lipid parameters to the pathogenesis of AMD. The aim was also to test the influence of gender, on lipo- and apoprotein values in AMD patients and the relation of lipoprotein parameter values and development of the early and advanced (late) form of the disease.

PATIENTS, MATERIALS AND METHODS

In a cross-sectional study conducted at the University Clinic of Ophthalmology, 82 patients with age-related macular degeneration, 70.3 ± 7.1 of age and 80 control subjects 69.32 ± 4.48 of age were included. Patients underwent complete ophthalmological examination including visual acuity assessment, colour fundus photography and fluorescein angiography. In order to analyze the influence of AMD type to lipoprotein and apolipoprotein parameters, the AMD patients were classified into 2 groups. According to their visual acuity, color fundus photography and fluorescein angiography, we defined (a) early AMD as the presence of multiple small drusen, few intermediate drusen or pigment abnormalities and large confluent drusen, multiple intermediate drusen and non-central geographic atrophy and (b) late (advanced) AMD as the presence of exudative AMD (choroidal neovascularization) or geographic atrophy involving the center. This classification was based on most severely affected eye (15).

They were thoroughly clinically examined and completed a questionnaire about their habits including BMI, physical activity, smoking etc. Out of a total number of the AMD patients, 61 were females (75.3%) and 21 (24.7%) were males. Eighteen cases (21.95%) had an early form of the disease while the rest of them (78.05%) had the advanced form.

The control subjects were selected from the employees of the Institute of Ophthalmology, Clinical Center of Serbia, Belgrade, and their relatives who were, without any signs of the acute conditions or maculopathy at the time of the study.

All the subjects consented to participate in the study. The local Ethic Committee approved this study. The blood samples for analysis were taken after 12-14 hours overnight fast. All the laboratory tests were done immediately. The lipid status was estimated on Olympus AU 400 biochemical analyzer, while apolipoprotein concentration was determined on Behring nephelometer using the immunochemical methods. The concentration of HDL₃-subfraction was measured using the spectrophotometric method after precipitation of LDL, VLDL and HDL₂ particles with 15% PEG solution (Polyethylene-glycol, Mm=20,000 D). The concentration of HDL₂-cholesterol was obtained by subtraction of HDL₃-cholesterol from total HDL-cholesterol obtained after precipitation of LDL and VLDL particles with 7.5% PEG and centrifugation 15 minutes at 3000 rpm. The concentration of LDL-cholesterol (LDL-C) was calculated by the Friedewald formula, but for samples with triglycerides (TG) concentration > 4.50 mmol/L, it was determined using direct enzymatic method.

Statistical analyses were performed by SPSS v.10.0 statistical package using Mann-Whitney U test, Student's t-test, Chi-Square, and ANOVA test. Results were presented as mean ± S.D. for continuous normally distributed variables, and as median and interquartile range for non-normally distributed data. Spearman's Rank correlation test was used to define correlations of the tested parameters between and within groups. All statistical tests were two-tailed. P values ≤0.05 were considered statistically significant.

RESULTS

Lipid and apolipoprotein values in patients with AMD and control group (CG) are presented in Table 1.

Table 1. Lipid and apolipoprotein parameters in AMD and control subjects

Parameter	AMD n=82	CG n=80	P
Apo A1 (g/L) ^a	1.69 ± 0.36	1.62 ± 0.32	0.192
Apo A2 (mg/L) ^a	358.1 ± 65.97	361.9 ± 65.75	0.723
Apo B (g/L) ^a	1.208 ± 0.287	1.18 ± 0.255	0.514
Apo E (mg/L) ^b	44.5 (38.05-49.05)	43.9 (36.15-53.87)	0.852
Lipoprotein (a) (g/L) ^b	0.098 (0.098-0.192)	0.098 (0.098-0.177)	0.476
Total cholesterol (mmol/L) ^a	6.25 ± 1.12	5.57 ± 0.84	<0.001
HDL-cholesterol (mmol/L) ^a	1.48 ± 0.33	1.43 ± 0.35	0.317
LDL-cholesterol (mmol/L) ^a	3.99 ± 1.10	3.60 ± 0.91	0.018
Triglycerides (mmol/L) ^a	1.56 ± 0.585	1.41 ± 0.528	0.091
HDL ₂ -cholesterol (mmol/L) ^a	0.379 ± 0.149	0.419 ± 0.192	0.218
HDL ₃ -cholesterol (mmol/L) ^a	0.883 ± 0.269	0.937 ± 0.242	0.233
non-HDL-cholesterol (mmol/L) ^a	4.77 ± 1.08	4.19 ± 0.82	<0.001

^a Arithmetic mean ± standard deviation (SD); ^b Median and interquartile

Significant difference of total cholesterol (P=0.000), non-HDL-cholesterol (P=0.000) and LDL-cholesterol values was found (P=0.018) in the group of AMD patients comparing to control subjects (Table 1).

Significant difference of total cholesterol values ($P<0.001$), HDL-cholesterol ($P<0.001$), HDL₃-cholesterol ($P=0.016$), LDL-cholesterol ($P=0.03$), and non-HDL-cholesterol ($P<0.001$), as well as apo A1 ($P=0.014$) and apo E values ($P=0.013$) was found between male and female AMD patients comparing to the adequate gender subgroup of the controls (Table 2).

Table 2. Lipid and apolipoprotein values in subgroups of tested males and females

Parameter	Males		Females		P
	AMD	CG	AMD	CG	
ApoA1 (g/L) ^a	1.57±0.36♣	1.55±0.33	1.75±0.35	1.71±0.29♦	0.014
ApoA2 (mg/L) ^a	338.4±54.5	359.9±66.2	365.6±68.8	362.0±65.5	0.484
ApoB (g/L) ^a	1.13±0.24	1.14±0.26	1.24±0.30	1.22±0.26	0.257
Apo E (mg/L) ^b	37.65♣ (30.6-44.1)	37.4 (36.3-43.1)	46.9 (43.2-48.5)	49.8♦ (45.8-54.8)	<0.001
Lipoprotein (a) (g/L) ^b	0.098 (0.098-0.189)	0.098 (0.098-0.101)	0.098 (0.098-0.164)	0.098 (0.098-0.146)	0.870
Total cholesterol (mmol/L) ^a	5.48±0.95♣	5.41±0.86	6.51±1.05	5.81±0.75♦	<0.001
HDL-cholesterol (mmol/L)	1.25±0.25♣	1.30±0.29	1.56±0.32	1.58±0.35♦	<0.001
HDL ₂ -cholesterol (mmol/L) ^a	0.32±0.11♣	0.42±0.18♣	0.41±0.16	0.41±0.2	0.269
HDL ₃ -cholesterol (mmol/L) ^a	0.72±0.12♣	0.95±0.23	0.95±0.29	0.93±0.26	0.016
LDL-cholesterol (mmol/L) ^a	3.53±0.84♣	3.45±0.88	4.15±1.15	3.80±0.93♦	0.003
non-HDL cholesterol (mmol/L) ^a	4.33±0.86♣	4.15±0.81	4.92±1.11	4.22±0.85	<0.001
Triglycerides (mmol/L) ^a	1.50±0.70	1.32±0.51	1.58±0.54	1.52±0.54	0.155

AMD- the patients with age-related macular degeneration; CG- The control group;

^a Arithmetic mean ± standard deviation (SD); ^b Median and 95% confidence interval for median;

♣ $P<0.05$, difference between AMD males and females

♦ $P<0.05$, difference between females and males in the CG

♣ $P<0.05$, difference between AMD males and males in the CG

Females with AMD had significantly higher apoA1 (P=0.039), apoE (P=0.002), total-cholesterol (P=0.000), LDL-cholesterol (P=0.026), total HDL-cholesterol (P=0.000), HDL₃-cholesterol (P=0.005) and non-HDL-cholesterol (P=0.029) comparing to males with AMD. Females in the control group had also higher values of apoA1 (P=0.023), total cholesterol (P=0.05), and total HDL-cholesterol (P=0.000) comparing to healthy males. The values of HDL₂ and HDL₃-cholesterol subfraction were similar in two gender control subgroups.

Serum lipid and apolipoprotein values in early and late form of AMD according to gender are presented in Table 3.

Table 3. Lipid and apolipoprotein values in subgroups of males and females with early and late form of AMD

Parameter	Males		Females		P
	EarlyAMD	Late AMD	Early AMD	Late AMD	
ApoA ₁ (g/L)	1.33 ± 1.09	1.60 ± 0.379	1.72 ± 0.337	1.75 ± 0.36	0.244
ApoA ₂ (mg/L)	337.0 ± 29.13	341.9 ± 57.49	370.37 ± 94.9	364.3 ± 61.1	0.607
Apo B (g/L)	0.83 ± 0.05●	1.18 ± 0.22	1.35 ± 0.32♥	1.21 ± 0.29	0.097
Apo E (mg/L)	40.0 ± 2.26	38.6 ± 10.7	50.87 ± 11.3	45.4 ± 8.18♠	0.011
Total cholesterol (mmol/L)	4.58 ± 0.085	5.60 ± 0.966	6.72 ± 0.941♥	6.44 ± 1.09♠	0.001
HDL-cholesterol (mmol/L)	1.18 ± 0.106	1.256 ± 0.27	1.50 ± 0.311	1.58 ± 0.33♠	0.002
HDL ₂ -cholesterol (mmol/L)	0.535 ± 0.035	0.34 ± 0.202	0.407 ± 0.126	0.399 ± 0.136	0.364
HDL ₃ -cholesterol (mmol/L)	1.21 ± 0.41●	0.789 ± 0.177	0.98 ± 0.253	0.911 ± 0.33	0.224
LDL-cholesterol (mmol/L)	2.79 ± 0.11●	3.63 ± 0.86	4.44 ± 1.14	4.05 ± 1.15	0.071
non-HDL ch (mmol/L)	5.18 ± 0.93	4.28 ± 0.82	5.08 ± 1.06	4.86 ± 1.14	0.125
Triglycerides (mmol/L)	1.23 ± 0.049	1.56 ± 0.73	1.78 ± 0.77♥	1.51 ± 0.43	0.359

● P<0.05, difference between males with early and late form

♥ P<0.05, difference between males and females with early form

♠ P<0.05, difference between females and males with the late form

Nevertheless, no significant difference was found between the early and advanced (late) form of AMD except for apo E, which was higher in the subgroup of early AMD (P=0.05); we found significant differences of tested parameters between males and females subgroups depending whether they had an early or advanced form of the disease. Significant difference of apo E (P=0.011), total cholesterol (P=0.001) and HDL-cholesterol (P=0.002) was found between males and females with the early and late form of AMD (Table 3). Apolipoprotein B (P=0.049), total

cholesterol ($P=0.006$) and total HDL-cholesterol values ($P=0.05$) were significantly higher in females with the early AMD comparing with males with the same stage of disease. Females with the advanced AMD had significantly higher apo E ($P=0.014$), total cholesterol ($P=0.006$), total HDL-cholesterol ($P=0.004$) and non-HDL-cholesterol ($P=0.054$) compared to males with the late form of AMD.

Males with the advanced AMD had higher values of apo B ($P=0.045$), lower levels of HDL₃-cholesterol ($P=0.028$) and were much younger ($P=0.029$) than males with the early form of disease.

The application of Spearman's correlation provided a series of very interesting correlations which were more numerous in the late form of disease. Besides usual and well-known correlations between lipo- and apolipoproteins, significant correlations were obtained between HDL cholesterol subfractions and other lipids and apolipoproteins. Accordingly, in the late form of disease, positive and significant correlation was obtained between total HDL cholesterol and HDL₃ cholesterol subfraction ($\rho=0.339$; $P=0.039$), HDL₂ and HDL₃ cholesterol subfractions ($\rho=0.454$; $P=0.006$), as well as between HDL₃ cholesterol subfraction and LDL cholesterol ($\rho=0.333$; $P=0.043$). It is worth mentioning that positive correlation between HDL₃ cholesterol concentration and patient age was obtained in the same group ($\rho=0.347$; $P=0.035$). Significant negative correlation was obtained between total HDL cholesterol and triglycerides ($\rho= -0.366$; $P=0.004$), and positive was found between triglyceride concentrations and apo E ($\rho=0.293$, $P=0.033$). High positive correlations were found between non-HDL cholesterol concentrations and total cholesterol ($\rho=0.802$; $P<0.001$), LDL cholesterol ($\rho=0.761$; $P<0.001$) and apo B ($\rho=0.737$; $P<0.001$).

DISCUSSION

Based on the obtained results, it may be concluded that patients with the age-related macular degeneration had significant disorder of lipid and apolipoproteins status compared to control group, indicating that this parameter disorder could have an important role in the pathogenesis of AMD.

Female AMD patients had significantly higher values of total cholesterol, total-HDL-cholesterol, non-HDL-cholesterol, LDL-cholesterol, HDL₂- and HDL₃-cholesterol values, compared to male AMD patients ($P<0.05$). Values of apoA1 and apoE were also significantly higher in female AMD patients compared to males with AMD. Females with the advanced form of AMD had significantly higher values of the atherogenic lipoproteins and apolipoproteins such as non-HDL-cholesterol, total cholesterol, and apoE. Although females with the advanced form of AMD had significantly higher HDL-cholesterol compared to males with the same form of disease, it turned out that more than 50% of total HDL-cholesterol concentration pertained to its proatherogenic HDL₃-cholesterol subfraction. The series of correlations set between tested parameters in the subgroups of males and females with the early and advanced form of AMD corroborated this finding. In the group of the advanced AMD, positive and significant correlations were obtained between HDL-cholesterol and atherogenic lipoproteins: LDL-cholesterol ($P=0.05$) and total cholesterol ($P<0.001$), especially between total HDL-cholesterol and HDL₃-cholesterol subfraction ($P=0.0339$), HDL₃-cholesterol and LDL-cholesterol ($P=0.043$), meaning that the increase of total cholesterol concentrations gives rise to elevation of LDL cholesterol, non-HDL cholesterol as well as total HDL cholesterol concentrations, before all, its proatherogenic HDL₃ subfraction.

These findings suggest that severe form of the disease is followed by the increase of atherogenic (non-protective) lipo- and apolipoproteins, and that females had higher values of these parameters compared to males with the same form of AMD.

A series of epidemiological studies have been carried out suggesting that estrogen deficiency in postmenopausal women may contribute to development and severity of AMD (16). Recently, the Women's Health Initiative Sight Exam Study on hormone therapy and AMD revealed that treatment with the conjugated equine estrogens and progesterone did not influence the occurrence of early AMD although it might reduce the risk of soft drusen or wet

AMD. Another large study found lower risk of large drusen but not AMD. Several other studies suggest that there is reduced risk of AMD with postmenopausal hormone replacement therapy. Estrogen physiologic effects are mediated by two estrogen receptor subtypes: ER α and ER β . Both subtypes are present and active in retinal pigmented epithelial cells (RPE) isolated from human males and females eyes. Cousins et al. (17) reported that estrogen deficiency in middle aged female C57B/6 mice led to increase Bruch's membrane thickening with sub-RPE deposit formation, and that aging females had worse deposits than aged males in models of both dry and wet AMD.

The increased values of atherogenic lipids and lipoproteins in AMD patients support the theory of correlation of AMD pathogenesis and atherosclerosis and accelerated pathogenesis of AMD in patients who also develop atherosclerotic process (18, 19).

The AMD literature is dominated by citations about large association between neovascularization and plasma cholesterol in the EDCC study (20) and neovascularization and apoB in the Beaver Dam Eye Study (BDES) (21). A total of 26 studies reporting data on plasma cholesterol and/or atherogenic lipoproteins, 5 of them showed positive association, 3 of them showed a negative association depending on the AMD parameter, and 15 showed no significant association (22).

The contribution of apoA1, as anti-atherogenic apolipoprotein to AMD has been examined less. (23). Elevated HDL-cholesterol levels are considered protective against cardiovascular disease, but, it might be surprising that out of 20 studies, 7 showed higher risk for AMD among patients with elevated HDL-cholesterol, 4 showed reduced risk, 8 showed no effect, and one showed higher and lower risk depending on the examined variable (21). None of these studies analyzed the values of HDL-cholesterol subfractions. Our examinations showed that regardless of similar values of total HDL cholesterol in AMD patients and control subjects, there was significant difference between the concentrations of HDL₂ and HDL₃ subfractions. AMD patients had significantly higher values of non atheroprotective HDL₃-cholesterol compared to control group. Females had significantly increased values of total, LDL-, HDL-cholesterol and non-HDL-cholesterol compared to males, but also including higher values of HDL₃-cholesterol. These findings can indicate the correlation between HDL₃ subfraction and atherosclerotic changes in elderly population and even the association with AMD development.

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DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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