

# Enhanced susceptibility of low-density lipoprotein to oxidation in wet type age-related macular degeneration in male patients

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## ABSTRACT

**Objectives:** To determine the susceptibility of low-density lipoprotein (LDL) to oxidation in the plasma of male patients with wet type age related macular degeneration (AMD) and in a similar control group, in order to evaluate the LDL oxidative status as risk factor of AMD.

**Methods:** We conducted this study in the Retina Service, Department of Ophthalmology, Nikookari Eye Hospital – Drug of Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran during the period between October 2004 and December 2005. Sixty male patients with AMD (mean age  $67 \pm 16$  years) with BMI  $4.1 \pm 1.3$  were selected as the patient group. The control group consisted of 60 males, apparently healthy, and without ophthalmologic signs and family history of AMD. Low-density lipoprotein was isolated by gradient ultracentrifugation and susceptibility of LDL to in vitro copper – mediated oxidation was assayed by measuring conjugated dienes production (lag phase duration) at 234 nm. Lipid and lipoproteins were determined by standard methods.

**Results:** Comparing with control, significant reduction in the duration of lag phase ( $p < 0.004$ ) and a significant increase in LDL-C concentrations ( $p = 0.006$ ), were noticed. No significant change in cholesterol ( $p > 0.3$ ), triglyceride ( $p > 0.1$ ) and high density lipoprotein cholesterol ( $p > 0.1$ ) levels were found between control and patient groups. A significant negative correlation between Lag phase and LDL-C levels ( $p = 0.004$ ,  $r = -0.364$ ) was found in the patient group.

**Conclusions:** The increased LDL concentration and enhanced susceptibility of LDL to oxidation may play a roll in the wet type AMD process.

*Saudi Med J 2007; Vol. 28 (2): 221-224*

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*Received 10th June 2006. Accepted 10th October 2006.*

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Age related macular degeneration (AMD) has been reported to be the leading cause of severe irreversible central vision loss and legal blindness in individuals 60 years of age or older.<sup>1-5</sup> The AMD can be divided into 2 main forms, non-neovascular (dry or non-exudative) and neovascular (wet or exudative).<sup>6,7</sup> Despite recent advances in the management of AMD, the majority of neovascular cases are not amenable to proven treatment. A possible precise recognition of the risk factors of AMD and proper prevention in those people can play a decisive role in preventing the development of AMD. At present, the origin of AMD remains unknown. Risk factors implicated in clinical and laboratory studies include drusen, genetic predisposition,<sup>8-11</sup> cigarette smoking,<sup>12</sup> post menopausal estrogen decrease,<sup>13</sup> cardiovascular risk factors (including systemic hypertension),<sup>14,15</sup> excessive exposure to sunlight,<sup>16</sup> and atherosclerosis.<sup>17</sup> It is based on mounting evidence that AMD shares both risk factors and pathogenetic mechanisms with atherosclerosis, resulting in the deposition of lipid in the sclera and in the Bruch membrane. There is evidence that the scleral lipid ultimately results in a decrease in choroidal blood flow as well as an elevation of choriocapillary pressure,<sup>18</sup> and the lipids in the Bruch membrane result in basal deposits and drusen and in calcification and fragmentation of the membrane.<sup>19,20</sup> Since oxidized low-density lipoprotein (LDL) has been recognized as playing an important role in the initiation and progression of atherosclerosis,<sup>21</sup> this study was therefore undertaken to determine the susceptibility of LDL to oxidation and its relation to other lipid and lipoproteins in wet type AMD.

**Methods.** We conducted this study in the Retina service, Department of Ophthalmology, Nikookari Eye Hospital – Drug of Applied

Research Center, Tabriz University of Medical Sciences, Tabriz, Iran during the period between October 2004 and December 2005. The participants of this study included 60 men aged 60-84 (mean age  $67 \pm 16$ ) with bilateral wet type AMD. Approval for the study was obtained from the ethical committee of Tabriz University of Medical Sciences, which is in compliance with the Helsinki Declaration. All patients were evaluated by a retina specialist. Wet type AMD was detected by slit-lamp examination with a 78-diopter indirect lens, fundus photography and fluorescein angiography (Imagenet 2000, Topcon TRC50IX, Topcon Corp, Japan). The control group consisted of 60 men aged 63-82 years (mean age  $73 \pm 6.3$ ) without ophthalmologic complications and family history of AMD. Exclusion criteria for the patient group were dry type AMD, diseases other than AMD associated with neovascularization and also cardiovascular, renal and liver diseases. None of the patients were on antioxidant micronutrient supplementation and they all lived in the same industrial area. Due to the rather small numbers of patients in the studied groups, we excluded diabetics and current or past smoking patients from the study. Both groups of patients were matched by age and gender. In all cases the diagnosis of wet type AMD was based on ophthalmoscopy signs of disease, fundus photography and fluorescein angiography of the retina. In all patients we evaluated the body mass index (BMI). After obtaining informed consent, fasting venous blood from each subject was collected in EDTA – coated tubes. To separate plasma, the blood was centrifuged at 3000 g for 10 minutes at 4°C. Total serum cholesterol (Cho), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) level were assayed by commercially available kits. The LDL fraction was isolated from plasma by ultracentrifugation (Beckman Optima TLX) at 15° at 100,000 rpm for 4 hours with the TLA-100.3 rotor. Oxidation of LDL (50 µgr) was determined as the production of conjugated dienes induced  $\text{Cu}^{2+}$  (5 µM) every 5 minutes at 234 nm in one ml phosphate buffer solution at 37°C at ultraviolet (Cecil 8000. spectrophotometer) and the results were recorded as lag phase.<sup>22,23</sup>

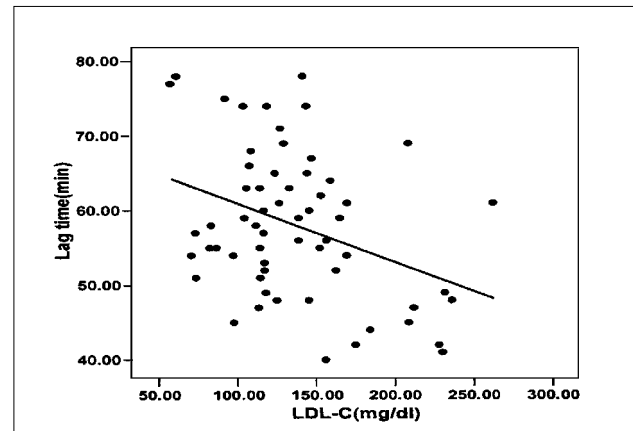
The statistical analysis was carried out using the SPSS 12 for windows program. Results are expressed as mean  $\pm$  SD. Independent-sample t-test and Mann-Whitney U test as appropriate were used to assess significance of differences between control and AMD patients. Correlation was evaluated by Pearson's test and the statistical significance was set at  $p < 0.05$ .

**Results.** Table 1 describes the biochemical investigations in the AMD and control patients. We

**Table 1** - Comparison of patients and control group in respect of LDL oxidation, lipid and lipoprotein profiles.

Investigations	Mean $\pm$ SD		
	Patients (n=60)	Control (n=60)	P-value
Cholesterol (mg/dl)	197.1 $\pm$ 45.6	190.1 $\pm$ 29.8	>0.3*
Triglyceride (mg/dl)	169.3 $\pm$ 85.7	151.3 $\pm$ 43	>0.1*
LDL-C (mg/dl)	137.4 $\pm$ 45.9	118.5 $\pm$ 24.7	=0.006**
HDL-C (mg/dl)	39.8 $\pm$ 9.6	42.4 $\pm$ 9.4	>0.1*
Lag time (min)	57.3 $\pm$ 12.1	61.7 $\pm$ 12	<0.04*

\*Independent-sample t-test; \*\*Mann-Whitney U test, LDL-C -low-density lipoprotein cholesterol, HDL-C- high density lipoprotein cholesterol



**Figure 1** - Correlation between the serum low-density lipoprotein (LDL-C) and Lag time of LDL oxidation in the patient group ( $r = -0.364$ ,  $p = 0.004$ ).

found a significant increase ( $p = 0.006$ ) in LDL-C and a significant decrease ( $p < 0.004$ ) in Lag phase in the patient group when compared with the control group. There were no significant difference in Cho ( $p > 0.3$ ), TG ( $p > 0.1$ ) and HDL-C ( $p > 0.1$ ) concentrations between control and patient groups. A significant negative correlation between Lag phase and LDL-C levels ( $p = 0.004$ ,  $r = -0.364$ ) was found in the patient group (Figure 1). In the AMD group, we found that an average BMI index ( $24.5 \pm 4.8 \text{ kg/m}^2$ ) was higher when compared with control patients ( $22.7 \pm 4.2 \text{ kg/m}^2$ ).

**Discussion.** All studies demonstrate that the prevalence, incidence, and progression of AMD rises steeply with increasing age,<sup>24,25</sup> but knowledge of other possible risk factors is controversial. It is possible that in different populations the relative role of individual risk factors may vary. It is difficult to interpret connections between lipid changes and development of AMD. Atherosclerotic vascular disease, due to influence on choroidal circulation, has been hypothesized as

possible pathogenetic factor for development of AMD. However, the study of the relationship of lipid change and atherosclerosis and the development of AMD has not presented uniform results.<sup>13,24-31</sup> Some studies have found an increased risk of AMD with a past cardiovascular event<sup>32,33</sup> systemic hypertension<sup>26,30,34</sup> and increased blood cholesterol levels,<sup>13,31</sup> although other studies have found no association with vascular events,<sup>28,31,35</sup> systemic hypertension<sup>28,31,35,36</sup> or blood lipid levels.<sup>28-35</sup>

Results of our study are consistent with results of previous studies correlating AMD with lipid disturbances; further, our results showed, in the AMD group, a significant correlation between Lag phase and other lipid factors such as TG, HDL and LDL. Hyman et al<sup>26</sup> found a positive association between neovascular AMD and higher cholesterol intake and elevated serum HDL-C. In this study AMD type was not related to serum cholesterol, TG and LDL-C. Similarly, a positive relationship was found with high serum HDL-C and an inverse with total cholesterol-HDL ratio by other authors.<sup>30</sup>

The interpretation of these results is difficult and inconsistent with the hypothesized connection of AMD to lipid changes and cardiovascular disease. Although scientific literature documented multiple etiologic theories and pathologic abnormalities in patients with AMD, blood lipid abnormality and atherosclerotic process could play an important role in AMD development by affecting the flow of choroidal vessels, but the mechanism for this process is unclear.<sup>27</sup> Some of the differences in results among the various studies may be due to differences in populations with completely different nutritional habits, or to methodological issues. One of the serious restrictions in establishing the role of lipid metabolism in the development of AMD is a lack of direct possibility for its measurement in the retinal vessels. In interpreting the results it must be assumed that the concentration in the peripheral blood correlates with the concentration in the eye.

Apart from genetic conditions, a very large group of risk factors is involved in the development of AMD. It seems that changes in lipid metabolism could play a pathogenic role, especially at the very beginning of natural history of the AMD development and could have a damaging influence also on choriocapillaris. It can be the reason for ischemia of the fovea avascular zones and disorders in the physiologic balances of angiogenic and antiangiogenic factors, which might consequently lead to neovascularization of the macular region.

It was concluded that patients with wet type AMD have an increased atherogenic tendency of serum lipids and an increased susceptibility of LDL to oxidation.

Our study confirmed that serum lipids and increased susceptibility of LDL to oxidation in such patients could be introduced as a satisfactory method in prognosis of wet type AMD. However, more research will have to be conducted to assess the significance of LDL susceptibility to oxidation in prognosis and development of wet type AMD.

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