

## Assessment of serum lipids in patients with age related macular degeneration from Pakistan

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### Abstract

**Objective:** To determine serum lipids in patients with age related macular degeneration from Pakistani population.

**Methods:** The study was a cross sectional, randomized and case-control. Selected subjects ages were  $\geq 50$  years and were normotensive, non-diabetic with no family history of any such disease and no complication of posterior ocular chamber other than age related macular degeneration (AMD). Controls were age matched healthy individuals with no symptoms of AMD. Diagnosis of AMD was done through conventional diagnostic techniques by professional ophthalmologists. Serum samples were analyzed for total cholesterol, triglycerides, LDL and HDL using commercially available kits. Data were compared with Student's t-test. Pearson correlation was calculated for relationship between different parameters.  $P < 0.05$  was considered significant.

**Results:** Compared to controls, AMD patients had significantly greater total cholesterol concentration ( $p < 0.041$ ), and power HDL/LDL ratio ( $p < 0.038$ ), while serum triglycerides, HDL and LDL were non-significantly different from control subjects. Total cholesterol in AMD patients was significantly correlated with TG, LDL and HDL ( $p < 0.0001$ ).

**Conclusion:** The study indicates that high cholesterol might be a predictor of AMD and can be a diagnostic parameter.

**Keywords:** AMD, Lipid profile, Pakistan. (JPMA 64: 664; 2014)

### Introduction

Age-related macular degeneration (AMD) has been a significant cause of blindness in elderly population and an issue for public health concern due to its high prevalence in aged people.<sup>1</sup> The causative factors of AMD include multiple genetic and environmental factors. Two types of AMD are recognized, "dry" and "wet". Early symptoms in both types of AMD include deposition of debris known as drusen on bruch's membrane (BM) and the appearance of extracellular matrix separating the choriocapillaries from the retinal pigment epithelium (RPE). Later stages of dry AMD involve the degeneration of photoreceptor and RPE cells resulting in geographic atrophy. In wet AMD, new blood vessels grow from the choriocapillaries through the BM known as choroidal neovascularization (CNV) that leads to visual loss. CNV occurs in 10-15% of AMD cases, yet it accounts for over 80% of debilitating visual loss in AMD patients.<sup>2</sup>

Normally, photoreceptors are maintained by the RPE, which is a metabolically active tissue. Oxidized lipids are taken up by the base of RPE cells.<sup>3</sup> It is likely that these cells are involved in clearance of the sub-retinal space from such lipid deposits.<sup>4</sup> Outer segments of

photoreceptors in RPE are rich in lipid content and these cells process lipids. Tremendous amount of energy is utilized by RPE cells to phagocytose and recycle lipids back to photoreceptors in order to preserve visual function. In fact, the RPE cycles approximately 30,000 lipid rich cells per day.<sup>5</sup>

With advancing age, the number of RPE cells decrease<sup>6</sup> creating an imbalance in this clearance mechanism ultimately leading to accumulation of lipoprotein and lipid-containing debris in sub-RPE and BM.<sup>7</sup> These particles accumulate in the inner collagenous layer and are present within the drusen, basal laminar and linear deposits; this is a critical event and a prominent histopathological marker in AMD.<sup>8</sup> This implicates that improper clearance of debris by the RPE is one of the factors to cause AMD symptoms.<sup>9</sup>

It has been shown that drusen found in AMD share a number of molecular constituents with atherosclerotic plaques. These include lipids and some proteins like vitronectin, apolipoprotein E, calcium and complement components demonstrating ultimate relevance between advanced AMD and carotid artery atherosclerosis. In addition both of these pathologies occur adjacent to an elastic layer, in the vicinity of vascular endothelial cells and pericytes.<sup>10</sup>

Controversies exist as regards the association of the AMD with serum lipid profile. One possible reason might be the

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different forms and stages of AMD in the considered subjects. van Leeuwen et al.<sup>11</sup> found association of elevated concentration of high density lipoprotein cholesterol (HDL) but not total cholesterol with an increased risk of non-vascular AMD. Similarly, no difference in total cholesterol, triglycerides, phospholipids, high and low density lipoprotein concentration was observed between AMD patients and controls in a study done by Abalain et al.<sup>12</sup> Likewise, in another study no significant difference could be found in the serum lipoprotein concentration between AMD patients and normal subjects.<sup>13</sup> Some correlations between serum lipid and AMD have however been shown in cases where the focus was high density lipoproteins. Two major studies done by van Leeuwen et al.<sup>11</sup> and Delcourt et al.<sup>14</sup> demonstrated that AMD was associated with elevated HDL levels. Contrary to this, Reynolds et al.<sup>15</sup> found that high levels of HDL were inversely correlated with advanced AMD indicating HDL to reduce the risk of wet type AMD.

The current study was designed and carried out to determine the association of total cholesterol and serum lipids with AMD in a sample from Pakistani AMD patients to compare with control subjects. To the best of authors' knowledge, the present is the first such report from Pakistan.

## Materials and Methods

The study was randomized and case-control. It was conducted between February 2010-11. The study design was approved by the "Ethics Committee for Research on Human Subjects", Al Shifa Trust Eye Hospital, Rawalpindi. Patients were screened for AMD, while healthy subjects were also screened for any related disease condition that could bias the results. Written informed consents were obtained from all patients as well as control subjects after explaining the study procedure to each one of them.

The patients were recruited from Al Shifa Trust Eye Hospital, Rawalpindi, Pakistan. A total of 3911 patients were examined. The inclusion criteria were: subjects of both genders aged 50 years or above, normotensive, non-diabetic, no family history of such diseases and no complication of posterior ocular chamber other than the AMD. Hospital staff workers and subjects from other OPDs having healthy profile were recruited as control subjects (n=100). Control subjects were also screened with the same inclusion criteria and in addition having no complication of posterior chamber and more specifically AMD.

Qualified and trained ophthalmologists screened the patients in the outpatients department (OPD) at Al-Shifa Trusts Eye Hospital, Rawalpindi, Pakistan, through

recommended diagnostic tests that included: dilated fundus examination, ocular coherent tomography and fluorescent fundus angiography. Aided and unaided visual acuity of all the subjects was tested through the Snellen's chart. Intraocular pressures were also measured. Family history for the presence of any systemic disease or AMD and history of smoking were recorded. The demographic profile and examination findings of all the subjects were entered in the study performa and later tabulated. Cubital blood was collected to obtain serum. Serum total cholesterol, HDL and triglycerides (TG) concentration was estimated on a Chemistry Analyzer (Selectra, XL, Netherlands) using the commercially available kits (Gesans productions, Italy) and following the manufacturer's methods and instructions. LDL was calculated by the formula:

$$TG/5 + HDL\text{-total cholesterol} = LDL$$

Data were computed through Statistical Package for Social Sciences (SPSS version 14.0, Chicago, Illinois, USA). Chi-square ( $\chi^2$ ) was applied to study the difference in non-parametric parameters between the control and diseased group as well as wet and dry AMD patient groups. Student's t-test was applied to compare the biochemical parameters of AMD patients against control subjects. Pearson product-moment correlation coefficient (PPMCC) was applied to correlate different symptoms of AMD with the lipid profile.  $P > 0.05$  was considered to be the significant difference.

## Results

Of 3911 patients only 90 (2.30%) were confirmed for the presence of AMD. AMD was diagnosed on appearance of any of the following symptoms; drusen, geographic atrophy, RPE atrophy, sub retinal neovascularized membrane exudation, haemorrhages, oedema and sub retinal CNV. Male to female ratio was 1.17:1 (55% males,

**Table-1:** Different parameters recorded in AMD patients compared to controls (mean  $\pm$  SD).

Parameter	Control (n=100)	AMD (n=90)	P value
Age (years)	63.49 $\pm$ 8.43	71.26 $\pm$ 10.08	0.448
IOP RE (mm Hg)	12.72 $\pm$ 2.28	14.48 $\pm$ 2.72	0.004**
IOP LE (mm Hg)	12.96 $\pm$ 2.05	14.28 $\pm$ 4.28	0.004**
Refractive equivalent RE (D)	-0.60 $\pm$ 3.49	-0.11 $\pm$ 2.38	0.354
Refractive equivalent LE (D)	-0.33 $\pm$ 3.73	-0.23 $\pm$ 2.11	0.847
Visual acuity unaided RE (LogMAR)	0.68 $\pm$ 0.29	0.80 $\pm$ 0.28	0.012*
Visual acuity unaided LE(LogMAR)	0.65 $\pm$ 0.23	0.85 $\pm$ 0.26	0.000***
Visual acuity corrected RE(LogMAR)	0.46 $\pm$ 0.37	0.68 $\pm$ 0.34	0.000***
Visual acuity corrected LE(LogMAR)	0.52 $\pm$ 0.27	0.77 $\pm$ 0.33	0.000***

\*\*\* P < 0.0001; \*\* P < 0.01; \* P < 0.05

IOP= Intraocular Pressure; RE= Right eye; LE=Left eye; SD= Standard deviation.



**Table-2:** Diagnostic findings in wet and dry AMD patient group.

Parameters	Wet AMD		Dry AMD (%)	
	$\chi^2$ value	P value	$\chi^2$ value	P value
Gender	5.918	0.015*	3.0	0.083
AMD pattern	32.947	0.000***	0.038	0.847
Small Drusen	NA	NA	NA	NA
Large Drusen	43.10	0.000***	8.33	0.004**
RPE changes	46.62	0.000***	9.846	0.002**
CNV	30.41	0.000***	0.926	0.336
GA	15.51	0.000***	0.926	0.336

\*\*P<0.01. NA= not available.  $\chi^2$  could not be calculated for small drusen as all the patients showed this basic diagnostic symptom for AMD.

CNV: Choroidal neovascularization. GA: Geographic Atrophy.

**Table-3:** Comparison of lipid profile of control patients to the AMD patients. (Mean  $\pm$  SD).

Parameters	Control (n=100)	AMD (n=90)	P value
Total Cholesterol	155.44 $\pm$ 41.02	166.18 $\pm$ 36.32	0.041*
Triglycerides	154.02 $\pm$ 97.63	164.68 $\pm$ 76.15	0.870
HDL	53.17 $\pm$ 14.97	57.48 $\pm$ 14.12	0.131
LDL	71.87 $\pm$ 34.44	75.76 $\pm$ 26.96	0.111
HDL/LDL	2.49 $\pm$ 7.72	0.92 $\pm$ 0.63	0.038*
Cholesterol/HDL	2.99 $\pm$ 0.64	2.98 $\pm$ 0.72	0.461
TG/HDL	2.84 $\pm$ 1.28	2.84 $\pm$ 1.07	0.733
LDL/HDL	1.44 $\pm$ 0.68	1.41 $\pm$ 0.67	0.525
LDL/cholesterol	0.44 $\pm$ 0.16	0.45 $\pm$ 0.12	0.459

\*p<0.05. SD= Standard deviation.

HDL: High Density Lipoprotein. LDL: Low Density Lipoprotein.

**Table-4:** Pearson correlation of different parameters in AMD patient group.

Parameter	Correlated to	R value	P value
Large drusen	RPE changes	0.281	0.010*
Small drusen	Large drusen	0.773	0.000***
CNV	Large Drusen	0.420	0.000***
	Pattern	0.433	0.000***
	Small druesn	0.582	0.000***
GA	CNV	-0.357	0.001**
TG	Cholesterol	0.575	0.000***
LDL	Cholesterol	0.685	0.000***
HDL	Cholesterol	0.646	0.000***
	TG	0.638	0.000***

\*\*\*P<0.0001. \*\*P<0.01. \*P<0.05.

r value= correlation coefficient. P value= level of significance.

CNV: Choroidal neovascularization. GA: Geographic Atrophy. TG: Triglycendis. LDL: Low Density Lipoprotein. HDL: High Density Lipoprotein.

large number of patients ( $p<0.0001$ ) in wet and dry AMD ( $p<0.002$ ). CNV and geographic atrophy was seen in significantly large number of wet AMD patients ( $p<0.0001$ ) (Table-2).

Total cholesterol levels in AMD patients were significantly higher as compared to the control subjects ( $p<0.041$ ). HDL/LDL ratio was significantly lower in AMD patient group ( $p<0.038$ ). Serum TG, LDL, HDL levels, and cholesterol/HDL, TG/HDL, LDL/HDL and LDL/cholesterol ratios did not show any significant differences from control subjects (Table-3). Cholesterol concentration showed significant positive correlation with TG ( $r=0.575$ ;  $p<0.001$ ), LDL ( $r=0.685$ ;  $p<0.001$ ) and HDL ( $r=0.646$ ;  $p<0.001$ ) respectively. Other correlations are shown in Table-4.

## Discussion

Age related macular degeneration is characterized by the occurrence of damage to the inner layers of retina as a result of the aging process. Causative factors underlying increased risk of AMD have although not been fully defined but deficiency of anti-oxidants, accumulation of toxic material in the RPE, genetic and some angiogenic factors are said to be involved in the emergence of AMD.<sup>16</sup>

In Pakistan the authentic data regarding AMD prevalence in the whole country is lacking. In a previous study by the same tertiary care hospital from where patients for the current study were recruited reported 55 AMD patients out of 1152 examined subjects, i.e., 6.74% prevalence with late AMD observed in only 0.86% patients. The figures justify the sample size for the present study.<sup>17</sup>

Extensive research in the western countries has been devoted to define the risk factors for AMD.<sup>18</sup> In comparison, literature from the eastern developing countries is scant, so much so that authentic reports on simple biochemical parameters in AMD cases are lacking. In our current data collection, 64% AMD patients were in the age range of 60-79 years, above this age the survival is relatively low as the average life expectancy in Pakistan is 66.71 years.<sup>19</sup> Some other studies have shown similar findings as the Beaver Dam Study.<sup>20</sup> Moreover, there was no gender difference in our population as far as the risk of AMD is concerned since the gender ratio was found to be similar between the control and the diseased group. In addition, no significant difference was found between the two genders in dry and wet type of AMD. By contrast, studies in other parts of the world have shown females to be at greater risk to develop AMD.<sup>21</sup> This deviation could have been due to the fact that females in Pakistan report less incidentally in the hospital due to a closed social set up. Presently, the number of patients having wet type AMD was much higher than the dry AMD, a finding compatible with those reported by Klaver et al.<sup>22</sup>

The major focus of the study was to analyze clinically significant association of lipid profile with AMD. None of

the subjects considered in the study was suffering from any systemic disease like hypertension and diabetes. As the current study showed significant elevation in serum total cholesterol concentration in AMD patients, our study predicts elevated cholesterol level to be a key risk factor for developing AMD. Reynolds et al.<sup>15</sup> demonstrated similar findings where higher total cholesterol was found to be associated with risk of AMD. Recently, Colak et al.<sup>23</sup> have also shown that elevated plasma lipid concentration could play an important role in the development of AMD in elderly people.

It is also been shown that cholesterol level rises due to a diet rich in saturated fats, smoking, sedentary life style and increased visceral fat.<sup>24</sup> Presently, care was taken to include subjects from the same ethnic background to circumvent this aspect in the assessment of lipid profile, such that the difference between the two groups was based only on the incidence of AMD in the diseased group. However, we cannot totally rule out the chances of some bias in the selection of subjects that could occur due to socioeconomic and demographic factors. Cases and controls were therefore screened from the same institute, and same geographical area, and with the same ophthalmologic procedures followed throughout.

Wet AMD has been previously associated to moderate and severe hypertension particularly in patients receiving antihypertensive treatment.<sup>25</sup> The present study is the first which reports higher cholesterol levels in the AMD patients compared to normal control subjects when all the considered subjects are normotensive.

The present study indicates that elevated cholesterol level in serum is a risk factor for AMD. Previous studies however show conflicting results in this regard whereby, a positive correlation of serum cholesterol and AMD was shown by the Eye Disease Case-Control Study Group<sup>9</sup> and by Belda et al.<sup>26</sup> Contrary to this some other studies found no association<sup>14</sup> or negative association as AMD patients had lower cholesterol levels in these reports.<sup>20</sup>

In the present study, LDL and HDL were found to be correlated with total cholesterol in AMD patients. It has been shown that lipids are deposited on the BM which may lead to neovascularization possibly by two mechanisms; one is direct as they release angiogenic cytokines, while the other is indirect through causing damage or death of RPE cells that are responsible to inhibit angiogenesis by producing inhibitory substances.<sup>27</sup>

Additionally, it is worth mentioning that there is inconsistency as regards the cholesterol lowering

medications in relation to AMD. Rotterdam and several other studies<sup>19,28</sup> did not find a relationship between cholesterol lowering medication including statins<sup>29</sup> and risk of AMD. In contrast, McGwin et al.<sup>30</sup> studied the same in a relatively larger population and found significant relationship of AMD and use of cholesterol lowering medications.

The present study reveals that this particular population of AMD patients, show elevation of cholesterol concentration and indicates high cholesterol concentration to be a causative factor for the development of AMD.

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