

Smoking, serum antioxidant vitamin levels and age-related macular degeneration

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Received: 2013-12-10 Accepted: 2014-04-04

吸烟,血清抗氧化维生素水平和老年性黄斑变性之间的关系

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摘要

目的:研究老年性黄斑变性(AMD)等级、血清抗氧化维生素水平(维生素A、C和E)和吸烟的关系。

方法:对84例患者行黄斑彩色眼底成像,根据AMD损伤率和损伤程度将其分为五组(等级I~V)。应用高效液相色谱法(HPLC)测量血清抗氧化维生素水平。根据吸烟状况分不吸烟者、已戒烟者和吸烟者三组,并统计吸烟者每年的吸烟总量。

结果:对照组中,维生素A、E、C水平分别为 0.874 ± 0.326 mg/L, 10.739 ± 4.874 mg/L和 1.737 ± 0.447 mg/L,AMD组中分别为 0.880 ± 0.305 mg/L, 9.487 ± 6.060 mg/L和 1.870 ± 2.191 mg/L,无显著差异($P > 0.05$)。AMD不同等级分组间维生素A($P = 0.881$)和E($P = 0.293$)水平差异无统计学意义,维生素C水平($P = 0.044$)随AMD程度的加深而增加。根据吸烟状况,AMD组与对照组之间无显著差异。根据每年的吸烟总量,两组之间有显著差异($P = 0.02$)。AMD越严重,年吸烟总量越高($P = 0.007$)。

结论:研究结果显示AMD与维生素A、E血清水平无关,但与维生素C的血清水平相关,并与吸烟量有关。

关键词:吸烟;抗氧化维生素;老年性黄斑变性

引用:Akkaya SC, Sahin O, Kazokoglu H, Tekeli F, Haklar G, Özkurt Y. 吸烟,血清抗氧化维生素水平和老年性黄斑变性之间的关系. 国际眼科杂志 2014;14(5):790-796

Abstract

• **AIM:** To evaluate associations between the grades of age related macular degeneration (AMD) and serum levels of antioxidant vitamins (vitamin A, C and E) and smoking.

• **METHODS:** Fifty-three AMD patients and 31 individuals having ages matching with the patient group were enrolled the study. Colored fundus photographs of the macula were used to place participants ($n = 84$) into one of the five groups (Grade I-V) based on the frequency and severity of the lesions associated with AMD. Serum antioxidant vitamin levels were measured using High Performance Liquid Chromatography (HPLC). Smoking status was classified as non-smoker, ex-smoker and current smoker. Total number of packs smoked per year, was defined.

• **RESULTS:** The distribution of vitamin A, E, and C levels were 0.874 ± 0.326 mg/L, 10.739 ± 4.874 mg/L, 1.737 ± 0.447 mg/L in control group and 0.880 ± 0.305 mg/L, 9.487 ± 6.060 mg/L, 1.870 ± 2.191 mg/L in AMD group, respectively. The difference between AMD and control group was not statistically significant for vitamin A, E and C levels ($P > 0.05$). There were no significant differences between subgroups of AMD for vitamin A ($P = 0.881$) and vitamin E ($P = 0.293$) but there was a contradicting rise of vitamin C levels ($P = 0.044$) with increasing levels of the disease. There were no significant differences between AMD and control group regarding smoking status, but there was a significant difference for total number of packs smoked per year ($P = 0.02$). An increase of number of total packs smoked per year was determined along with the rising grade of AMD ($P = 0.007$).

• **CONCLUSION:** We found no relation between AMD and serum levels of vitamin A and E but vitamin C levels was increase with AMD grades unexpectedly. We found dose-response relationship between smoking and AMD.

• **KEYWORDS:** smoking; antioxidant vitamin; age related macular degeneration

DOI:10.3980/j.issn.1672-5123.2014.05.02

Citation:Akkaya SC, Sahin O, Kazokoglu H, Tekeli F, Haklar G, Özkurt Y. Smoking, serum antioxidant vitamin levels and age-related macular degeneration. *Guoji Yanke Zazhi (Int Eye Sci)* 2014;14(5):790-796

INTRODUCTION

Age related macular degeneration (AMD) affect Bruch's membrane, choriocapillaris and retinal pigment epithelium. It is of progressive, degenerative and bilateral character^[1,2]. The prevalence of AMD is 1.7% in the population^[3]. AMD is the leading cause of blind registration in the western world, and its prevalence is likely to rise as a consequence of increasing longevity^[1,2].

The exact etiology of AMD is not known. During recent years, oxidative stress has been implicated in many disease processes, especially for age – related disorders and the pathogenesis of the AMD. One prominent hypothesis of aging proposes that oxidative damage to cells, initiated by free radicals, is an important cause of the aging process. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, high proportion of polyunsaturated fatty acids, and exposure to visible light. Among the risk factors, genetic defect for antioxidant enzymes (peroxidase, superoxide dismutase), decreased dietary consumption of antioxidant vitamins, and environmental factors (smoking) have been implicated. The extent of the tissue damage is the result of the balance between the generated free radicals and the antioxidant protective defense system^[4-10].

In this study we investigated the relations between smoking status, blood antioxidant vitamin levels, and grade of AMD.

SUBJECTS AND METHODS

In the present cross – sectional study, 53 patients who were aged 50 or more and had been followed up in the retinal unit due to AMD of various grades between February 2006 and June 2006, were enrolled alongside 31 individuals having ages matching with the patient group who had come to our polyclinic. Patients who had pathologies such as degenerative myopia, traumatic choroidal rupture, ocular histoplasmosis which could lead to CVNM and ones who were using vitamin preparations, were not included in the study.

Smoking status at the time of the examination was determined as follows: nonsmoker, if one had smoked less than 100 cigarettes in his/her lifetime; ex-smoker, if one had smoked more than 100 number of cigarettes in his/her lifetime but had stopped smoking before the examination; current smoker if one had not stopped smoking. Amount of total pack/years smoked was defined as the number of cigarettes smoked per day divided by 20, multiplied by the number of years smoked.

BMI's of the enrolled individuals in patient and control groups, were measured by determining their weight and height.

Ophtalmologic Examination and Determination of Grades

Complete ophtalmologic examination was performed on both patient and control groups. After determining the best corrected visual acuity of individuals, 1% tropicamide was applied to dilate the eyes of people in patient and control groups. Indirect ophtalmoscopic examination was carried out by a Volk lense of 90D, and fundus was photographed. During grading of the patients, AREDS grading system was

employed^[6,7]. In patients who had different AMD grade for each eye, the results of the eye with a higher grade was included in the study.

Biochemical Measurements Values for parameters such as vitamin A, vitamin E, vitamin C, hemoglobin, fasting blood sugar, cholesterol, triglyceride, HDL, LDL, VLDL, were investigated for patient and control groups.

Blood samples of patient and control groups were obtained after at least 8 hours of a fasting period, and then stored in a refrigerator at -20°C . After obtaining blood samples of all the individuals in patient and control groups, biochemical analysis was performed on the same day.

Measurement of Total Cholesterol, Triglyceride, High Density Lipoprotein, Low Density Lipoprotein and Very Low Density Lipoprotein in Serum Measurements were carried out by calorimetric method in a Dax 48 Tecnicon autoanalyzer and kits belonging to Biocon company were used. Normal range of values were as follows: total cholesterol 100–200mg/dL, triglyceride 30 – 150mg/dL, HDL > 65mg/dL, LDL < 130mg/dL.

Measurement of Serum Vitamin A and E Values Reverse-phase HPLC and UV detection methods were employed for biochemical assays. Chromatographic system was comprised of Thermo Finnigan Spectra Model high performance liquid chromatography (HPLC) device, P1000 pump, AS3000 autosampler, Spectra UV1000 UV detector, SCM1000 degasser unit and SN4000 system control unit. Moreover, a column calibrated to 30° was employed. For mobile phase, isocratic elution was performed at 295 and 325nm with Recipe Vitamin A–E HPLC Detection Kit at 1.5mL/min flow rate. During measurements, α -tocopherol for vitamin E and all-trans-retinol for vitamin A, were used as a standard.

While the Level 1 values of the control used for vitamin A ranged between 0.78 and 1.08, the mean value was 0.90mg/L. Level 2 values of the control varied between 1.18 and 1.76mg/L, and the mean value was 1.47mg/L. Level 1 values of the control employed for vitamin E ranged between 12.2 and 18.2, and the mean value was 15.2. Level 2 values of the control varied between 20.0 and 30.0, and the mean value was 25.0.

Measurement of Serum Vitamin C Value Reverse-phase HPLC and ultraviolet (UV) detection methods were employed for measuring serum Vitamin C levels. The device mentioned above was used for the measurements along with using 125mm \times 4mm and 5 μm Bischoff Prontosil AQ columns. Isocratic elution was used with methanol at 0.75mL/min flow rate as mobile phase. Level 1 values for the control used for vitamin C, varied between 2.6 and 5.4mg/L, but the mean value was 4.0mg/L. The values of control for Level 2 ranged between 13.9–23.1, and the mean value was 18.5mg/L.

Statistical Analysis The comparisons between patient and control groups and AMD grades were carried out according to the distribution characteristics by employing parametric and nonparametric tests. Results were presented as mean \pm standard deviation values. Correlations between vitamin values and

biochemical parameters of patient and control groups, and AMD grades; were evaluated by using Pearson or Spearman correlation coefficient, if applicable. Values found to be significant with vitamin values in univariate analysis, were corrected by employing general linear model. The distribution among the corrected values was compared by again applying One – way ANOVA test. $P < 0.05$ was recognized as statistically significant. SPSS/PC 10.0 (SPSS Inc. Chicago, IL) was used for statistical evaluations.

RESULTS

Control group was consisted of 31 individuals; while 26 had complete vision, 4 had 0.8, and 1 had 0.7 vision. Senile cataract was the reason behind the lesser degree of vision in those 5 individuals. A description of study participants is given in Table 1. The difference between AMD and control group was not statistically significant regarding these features. Smoking status in control and AMD groups, is given in Table 2. While there was no significant difference between the 2 groups in terms of smoking status ($P=0.09$), there was a significant difference between them regarding the amount of cigarettes smoked ($P=0.02$).

In all cases, correlations between vitamin A, E, and C levels, and demographic and biochemical values, were investigated. While no correlation was found between serum vitamin A level and the investigated values, a statistically significant correlation was detected between serum vitamin E level, and Hg ($r=0.25$), and serum Cho. level ($r=0.22, P=0.04$), whereas serum vitamin C level showed statistically significant correlation with age ($r=0.26, P=0.01$). Corrected results of the vitamins were employed in the assessments.

Mean values of vitamin A, E, C measured in patient and control groups, are shown in Table 3. No statistically significant difference was found between patient and control groups regarding vitamin A ($P=0.932$), vitamin E ($P=0.330$), and vitamin C ($P=0.797$) values. AMD group was consisted of 53 people. While 12 had complete vision, 2 had 0.9 vision, 9 had 0.8 vision, 10 had 0.7, 4 had 0.6, 1 had 0.4, 2 had 0.3, 1 had 0.2, 1 had 0.16, 2 had 0.05, and 9 had <0.05 vision.

Demographic characteristics, biochemical values, and accompanying risk factors for AMD patients regarding the grades, are shown in Table 4.

No statistically significant difference was found between the grades regarding all the parameters except age, whereas the age difference between grade 2 and 3, and grade 2 and 4, were statistically significant ($P=0.002$). Smoking status distribution among AMD group according to the grades, is shown in Table 5.

While there was no significant difference between AMD grades in terms of smoking status ($\kappa^2 = 0.441$), the difference between grades regarding amount of smoked cigarettes, was determined to be significant ($P=0.007$). There were significant differences between grade 3 and 4, grade 3 and 5, grade 2 and 4, and grade 2 and 5. Serum vitamin A, E, C levels of AMD group in terms of grades, are shown in Table 6.

Table 1 Baseline characteristics of participants in the control and AMD group

Variable	Control group (n=31)	AMD group (n=53)	¹ P
Age (a)	69.77±8.01	72.36±9.63	0.21
Sex			
Male	11 (35.5%)	24 (45.3%)	² 0.49
Female	20 (64.5%)	29 (54.7%)	
BMI (kg/m ²)	29.26±4.25	27.49±5.11	0.11
Hg (g/dL)	13.07±1.50	13.24±1.47	0.60
FBS (mg/dL)	109.64±29.50	110.62±27.39	0.87
Chol (mg/dL)	197.97±39.59	210.21±38.16	0.16
TG (mg/dL)	124.19±42.95	129.40±70.05	0.70
HDL (mg/dL)	57.64±14.98	60.26±13.74	0.41
LDL (mg/dL)	115.03±37.38	123.44±31.98	0.27
VLDL(mg/dL)	25.04±8.56	25.98±11.80	0.70
HT			
Present	23 (74.2%)	37 (69.8%)	0.80
Absent	8 (25.8%)	16 (30.2%)	
DM			
Present	11 (35.5%)	14 (26.4%)	0.46
Absent	20 (64.5%)	39 (73.6%)	

BMI: Body mass index, Hg: Hemoglobin, FBS: Fasting blood sugar, Chol: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, HT: Hypertension, DM: Diabetes Mellitus; ¹ t-test, ² Chi-square test.

Table 2 Cigarette smoking status in control and AMD groups

Smoking status	Control group n=31 (%)	AMD group n=53 (%)	² P
Non-smoker	15 (48.4%)	26 (49.1%)	0.99
Ex-smoker	14 (45.2%)	24 (45.3%)	
Current smoker	2 (6.5%)	3 (5.7%)	¹ 0.02
Pack/a	7.20	16.78	

¹ t-test, ² Chi-square test.

Table 3 Plasma vitamin A, E, C levels of control and AMD groups

Variable	Control group (n=31)	AMD group (n=53)	¹ P
Vitamin A (mg/L)	0.874±0.326	0.880±0.305	0.932
Vitamin E (mg/L)	9.70±1.27	10.09±1.23	0.17
Vitamin C (mg/L)	1.73±0.44	1.50±0.55	0.16

¹ t-test.

While there was no significant difference between AMD grades, and vitamin A ($P=0.881$) and E ($P=0.28$); the difference of vitamin C level ($P=0.881$) was statistically significant between grade 1 and 4, grade 2 and 3, and grade 2 and 4.

DISCUSSION

Previous studies have shown that age is a risk factor in development of AMD^[11,12]. For example, Hirvela *et al*^[11] conducted an epidemiologic study on 560 people and after investigating several risk factors such as age, hypertension,

Table 4 Baseline characteristics in all categories of AMD group

Variable	Grade 2 n=16	Grade 3 n=15	Grade 4 n=7	Grade 5 n=15	P
Age	65.68±9.97	74.47±9.20	80.00±5.54	73.80±7.27	0.002
Sex					
M	6 (37.5%)	7 (46.7%)	4 (57.1%)	7 (46.7%)	0.809
F	10	8	3	8	
BMI	28.40±5.88	27.17±4.42	24.21±2.67	28.39±5.50	0.139
Hg	13.48±1.64	13.06±1.42	12.37±1.82	13.58±1.05	0.39
FBS	104.25±27.52	116.20±30.82	92.43±8.04	120.33±25.52	0.18
Chol	223.81±41.07	205.80±45.61	196.29±26.75	206.60±29.53	0.27
TG	135.50±68.50	147.93±98.03	92.57±24.58	121.53±46.63	0.35
HDL	59.75±17.36	59.46±12.20	60.14±12.55	61.66±12.58	0.93
LDL	133.56±35.84	122.00±32.53	114.71±28.70	118.15±28.70	0.49
VLDL	31.06±14.07	25.13±11.78	19.29±5.56	24.53±9.89	0.14
HT present	11 (68.8%)	12 (80.0%)	3 (42.9%)	11 (73.3%)	0.472
DM present	3 (18.8%)	6 (40.0%)	–	5 (33.3%)	0.265

Table 5 Smoking status in all categories of AMD groups

Smokingstatus	Category 2 n=16(%)	Category 3 n=15(%)	Category 4 n=7(%)	Category5 n=15(%)	¹ P
Non-smoker	9(56.3%)	10(66.7%)	2(28.6%)	5(33.3%)	² 0.441
Ex-smoker	5(31.3%)	5(33.3%)	5(71.4%)	9(60.0%)	
Currentsmoker	2(12.5%)	0(0%)	0(0%)	1(6.7%)	³ 0.007
Pack/a	12.28	9.58	25.59	24.67	

¹Kruskal Wallis test, ²Chi-square, ³Statistically significant.

Table 6 Plasma vitamin A, E, C levels of all AMD groups

Variable	Category 1 n=31	Category 2 n=16	Category 3 n=15	Category 4 n=7	Category 5 n=15	¹ P
Vit. A (mg/L)	0.874±0.326	0.815±0.213	0.924±0.505	0.934±0.129	0.879±0.160	0.881
Vit. E (mg/L)	9.81±2.06	10.23±1.91	10.06±1.79	10.39±1.41	9.60±1.64	0.28
Vit. C (mg/L)	1.73±0.44	1.51±0.55	1.99±0.51	2.31±0.31	1.96±0.40	² 0.002

Vit. : Vitamin; ¹One-way ANOVA, ²Statistically significant.

diabetes, smoking, myopia, they found only age to have an influence over AMD development. Another BDES study performed by Klein *et al*^[12] which had the title of “Five-year incidence of age-related macular degeneration”, underscored the importance of age both in early and late stage AMD.

In the present study, we determined age as an effective risk factor for grade of AMD disease. While mean age of AMD patients and control group, did not show any statistically significant difference, the difference between grades in the patient group was found to be statistically significant ($P = 0.002$). The age differences between grade 2 (mean 65.68) and grade 3 (mean 74.47), and between grade 2 and grade 4 (mean 80.00) were statistically significant.

Many epidemiologic studies showed smoking as an important risk factor for AMD^[13-24]. Several studies yielded results which indicated the influence of smoking was dependent on dose^[16]. Beaver Dam Eye Study showed that smoking elevates exudative AMD. Exudative AMD was found to have a 2.5 times higher incidence among current smokers compared to those of ex-smokers or nonsmokers (relative risk 2.5; 95% CI 1.01–6.20). Among males, exudative AMD risk in current smokers was found to be 3.29 times more than ex-smokers or nonsmokers (relative risk 3.29, 95% CI: 1.03–

10.50). POLA study showed late stage AMD risk as 3.6 times higher among current smokers compared to those of nonsmokers.^[14] Rotterdam Eye Study performed in Holland found the neovascular AMD risk among current smokers who were below 85 age, as 6.6 times higher than that of nonsmokers. Moreover, neovascular AMD risk has been found to be 3.2 times higher among ex-smokers compared to that of nonsmokers. The evaluation of the amount of cigarettes smoked, showed a significant elevation in AMD development risk for people who smoke 10 packs or more per year (relative risk 6.5; 95% CI: 2.9–14.8)^[16]. Furthermore, smoking is thought to have an influence over disease development due to its effects such as decreasing serum antioxidant level, reducing choroidal blood flow or increasing atherosclerosis^[16]. A study conducted by AREDS in 2005, found correlation between smoking, and neovascular AMD (OR: 1.55, 95% CI: 1.15–2.09) and central geographic atrophy (OR: 1.82, 95% CI: 1.25–2.65) depending on the dose (>10 pack/year)^[17].

In the present study, no difference was found between control and patient groups regarding number of current smokers. While 45.3% of AMD group was ex-smoker, 5.7% was current smoker. The 45.2% of control group was ex-smoker,

whereas 6.5% was current smoker ($\kappa^2 = 0.99$). However, regarding the evaluation of the amount of smoked cigarettes in terms of pack/year, there was a significant difference between the patient and control groups. While the mean amount of smoked cigarettes was 16.78/pack/year in AMD group, it was 7.20 pack/year in control group ($P = 0.02$). An assessment carried out between grades of AMD group in smokers, ex-smokers, and nonsmokers; but no significant difference was found between them ($P = 0.99$), however, there was a significant difference in terms of the amount of cigarette smokers in pack/year ($P = 0.007$). While the rate was 12.28 pack/year in stage 2, it was 9.58 pack/year in grade 3. Grade 4 had a rate of 25.59 pack/year and grade 5 had a rate of 24.67 pack/year. The amount of cigarettes smoked in late stage AMD (grade 4 and 5), was significantly higher than that of early stage AMD (grade 2 and 3) ($P = 0.007$). This result suggested that the influence of smoking was dependent on the dose and had a correlation with the severity of the disease.

Studies performed to show the association between AMD and antioxidant vitamins^[25-31], yielded different results. While some showed no association^[32,33], some showed antioxidant vitamins as protective against wet type AMD^[4,34,35].

EDCC study found a link between vitamin A and AMD^[34]. EDCC carried out the investigation on that manner between 1986-1990. 421 neovascular AMD patient (mean age 71) and 615 (mean age 68) control group were enrolled in the study. Serum carotenoid, vitamin C, vitamin E, and selenium values of patient and control groups were compared. Serum vitamin A, E, C values were measured as $\mu\text{mol/L}$ and classified as low, medium, and high. Neovascular AMD risk in the group which had a medium and high serum carotenoid levels (Vitamin A), was found to be significantly lower than the group that had a lower serum carotenoid level. While medium carotenoid level reduced the neovascular AMD risk to 50%, high carotenoid level decreased this risk to 30%. However, no significant difference could have been found in comparisons made between values of serum vitamin C, vitamin E, and selenium^[34].

The Blues Mountains Eye Study (BMES) conducted by Smith *et al*^[33] did not find any association between antioxidant level and AMD, as well. The evaluation of the results showed no significant difference between antioxidant or supplemental vitamins taken by diet and early or late stage AMD^[33].

In the present study, similar to the other studies (POLA, BLSA, BDES, BMES) in the literature except EDCC, no link was found between vitamin A and AMD. While the vitamin A value of control group was $0.874 \pm 0.326 \text{mg/L}$, it was $0.880 \pm 0.305 \text{mg/L}$ for AMD group. The comparison of AMD and control groups revealed no statistically significant difference in terms of vitamin A values ($P = 0.932$).

The distribution of vitamin A among grade 2-5, were as follows: $0.815 \pm 0.213 \text{mg/L}$, $0.924 \pm 0.505 \text{mg/L}$, $0.934 \pm 0.129 \text{mg/L}$, $0.879 \pm 0.160 \text{mg/L}$ respectively; and there was no significant difference between them ($P = 0.881$).

Two studies found an association between vitamin E and AMD: POLA and BLSA^[4,19].

The first study which found a link between vitamin E and AMD, was the POLA study conducted by Delcourt *et al*^[4] in France. They detected a weak negative relationship between vitamin E (serum α -tocopherol) level and late stage AMD ($P = 0.07$). However, after making an adjustment according to the serum lipid level, a significant negative link has been found between serum α -tocopherol level and late stage AMD ($P = 0.003$). Late stage AMD risk in the group with high vitamin E level, was found to be 82% lower than that observed in the group with low vitamin E level. Moreover, a significant correlation has been found between vitamin E level adjusted according to the lipid level and early findings of AMD ($P = 0.03$). However, no significant correlation has been determined between serum vitamin A and vitamin C levels, and AMD findings^[4].

The second study showing the protective nature of vitamin E over AMD, was performed by West *et al*^[36] between 1988-1990 on 976 patients selected among a BLSA population. According to their fundus photographs, patients were grouped in two as mentioned above: early stage AMD and late stage AMD. In the end of the study, α -tocopherol was found to have a statistically significant protective property against early stage AMD; while it was protective against the late stage too, this was not statistically significant. Moreover, the assessment of α -tocopherol (vitamin E), ascorbic acid (vitamin C), and β -carotene, all of which have been mentioned as antioxidant index, were found to be protective against early stage AMD. However, supplemental vitamin use was not determined to be a significant protective factor against AMD (OR = 0.9, 95% CI: 0.6-1.3)^[36].

Studies other than POLA and BLSA, showed no association between vitamin E and AMD^[32-34,37-39].

One of the studies which shows no association between vitamin E and AMD, is the case-control study performed by Perlman *et al*^[37] which categorized AMD as early and late stage. In the present study, we divided AMD into 4 grades and therefore, had the chance to evaluate in a more detailed manner. Their study found mean serum vitamin E level to be lower in the group with wet type AMD ($P = 0.03$). However, when an adjustment was made for vitamin E based on the serum cholesterol levels, the difference was determined to be insignificant.

In the present study, we detected no link between serum vitamin E levels and AMD. While vitamin E was $10.739 \pm 4.874 \text{mg/L}$ in the control group, it was $9.487 \pm 6.060 \text{mg/L}$ in the AMD group. The difference of vitamin E values between patient and control groups was not statistically significant ($P = 0.330$).

The correlation of serum vitamin E level with the cholesterol level was found to be significant ($r = 0.22$, $P = 0.04$). Vitamin E level adjusted according to the cholesterol value, was found to be $9.70 \pm 1.27 \text{mg/L}$ and $10.09 \pm 1.23 \text{mg/L}$ in control and AMD groups, respectively. The difference

between the control group and the AMD group was not significant again ($P=0.330$).

The distributions of vitamin E between grades 2–5 were as follows: 7.414 ± 6.965 mg/L; 9.951 ± 6.387 mg/L; 12.240 ± 5.500 mg/L; 9.949 ± 5.653 mg/L; and the differences between them were not statistically significant ($P=0.293$). The differences between vitamin E values adjusted according to the cholesterol values in AMD grades, were again statistically insignificant ($P=0.28$).

Regarding the studies carried out to show the association between AMD and vitamin C, it can be observed that no significant correlation could have been found^[4,33,34,36,38]. In the present study, we did not detect any significant correlation between AMD and vitamin C, as well. Vitamin C levels between control and AMD groups were 1.737 ± 0.447 mg/L; 1.870 ± 2.191 mg/L, respectively; and the difference between them was not statistically significant ($P=0.797$). In our study, the correlation between serum vitamin C level and age, was found to be significant. Vitamin C level adjusted according to the age was 1.73 ± 0.44 mg/L in control group and 1.50 ± 0.55 mg/L in AMD group. The difference between AMD and control group was not significant again ($P=0.16$). In the present study, contrary to the general expectation which predicts vitamin C level to drop as the grade of the disease advances, it was found to be higher in advanced grades ($P=0.002$).

Although there are many studies which did not show any link between antioxidant vitamins and AMD, because it had decreased the loss of vision and progression of the disease in a placebo-controlled, double-blind clinical study of AREDS conducted between 1992–1998, AREDS recommends high-dose antioxidant vitamin (50mg vitamin C, 400IU vitamin E, 15mg β -carotene) and 80mg Zn^[30,40]. Since it is not possible to determine the antioxidant agents in retina in vivo, we can measure their serum levels. However, by those instantaneous measurements, we can not know if the patient has adequate antioxidant throughout one's life. Particularly in elderly individuals in whom any deficiency is not observed, prescribing excessive vitamin and minerals seems risky to us since the side effects and indications have not been clearly outlined yet.

In light of those results, we believe that the role which deficiency of antioxidant vitamins play over etiology and grading of AMD, is not of primary character. However, the fact that our study was more cross-sectional than prospective, and that antioxidant vitamin values measured once might not show a direct correlation with the AMD findings occurring as a result of a changes throughout a lifetime, were the limitations of our study. The present study rather provides us information on vitamin values observed in AMD group alongside suggesting higher incidence of advanced grades in smoking individuals.

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