Documenta Ophthalmologica **81**: 387–400, 1992.

Serum levels of antioxidants and age-related macular degeneration

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Accepted 9 September 1992

Key words: Age-related Macular Degeneration, Selenium, Vitamin E.

Abstract. A number of reports have suggested that oxidative damage in the retina may contribute to the pathogenesis of Age-related macular degeneration (AMD). The present study was designed to investigate the hypothesis that serum levels of the antioxidants, Vitamin E and selenium are related to the pathogenesis of AMD. Fasting bloods were obtained from 80 patients with AMD and 86 controls. Assays for serum levels of Vitamin E, selenium, cholesterol and triglycerides were performed. Assessment of patients and controls was based upon eye examination, fundus photography and medical history. No significant difference was found in serum levels of Vitamin E between subjects and controls, however, there was a borderline association between AMD and both serum selenium levels and current smoking status. The results suggest that if oxidative damage is a factor in the pathogenesis of AMD, it is not reflected in serum levels of Vitamin E; further studies are required to clarify the possible relationship between serum selenium levels, smoking and AMD.

Introduction

The last decade has seen increasing interest develop in the possible role of oxidative damage in ageing and age-related diseases, including age-related macular degeneration (AMD). Oxidative damage occurs in body components as a result of the formation of oxygen free radicals from metabolic processes. It has been suggested that the retina is susceptible to oxidative damage due to a high content of polyunsaturated fatty acids, constant exposure to light and a high metabolic rate facilitating the production of free radicals [1]. Antioxidants such as vitamin E and glutathione peroxidase may play a role in reducing oxidative damage.

Although there is inconclusive evidence that oxidative damage is associated with AMD, vitamin E is being prescribed for AMD by some ophthalmologists. Vitamin E is found in the retina in a high concentration [2–4] which increases with age in humans [5]. The speculation that vitamin E may have an antioxidant role in the retina is supported by animal studies in which lipofuscin accumulation in the RPE occurs in vitamin E and antioxid-

ant deficiency [6–10]. Light adaptation has been shown to vary the vitamin E content of the RPE-choroid-scleral complex [11].

It has been proposed that light-induced free radical damage may be a factor in the aetiology of AMD [12]. However, the role of vitamin E in preventing light damage to the retina has not been substantiated by animal experiments in which supplementation of the diet with vitamin E has not decreased the light-induced retinal damage either in terms of electrical changes or histopathological changes [13, 14]. Studies in humans are limited; there has to date been no prospective study on the effect of treating AMD patients with vitamin E. An earlier study examining risk factors in AMD, including serum vitamin E levels, failed to detect any difference between cases and controls [15]. However, the study was limited by the small number of cases and only choroidal neovascularisation was studied.

Studies of serum selenium and vitamin E have been previously performed in other diseases in which oxidative damage has been implicated, such as cardiovascular disease and cancer [16–18]. Vitamin E scavenges free radicals [19–21] while glutathione peroxidase, of which selenium is the prosthetic group, reduces hydroperoxides formed by the scavenging action of vitamin E [22]. Their antioxidant actions are complementary to each other [23]. The present study investigates serum vitamin E and selenium levels in both atrophic and neovascular forms of AMD.

Subjects and methods

Cases and controls were recruited from the Sydney Eye Hospital outpatient clinics. The sources for the cases and controls were as follows:

Sources of cases

1) Follow-up patients from a previous study on serum anti-retinal auto-antibodies in AMD from 1986–1989 [24]. These patients were previously recruited from clinics at the Sydney Eye Hospital. Patients who were on vitamin E supplements were included except for those who only started using the supplements after the diagnosis of AMD had been made since the serum vitamin E levels of these patients would be expected to be different from those at the time of diagnosis.
2) Patients recruited from the outpatient clinics at Sydney Eye Hospital in 1989.
3) Persons introduced by patients as 'controls' who were subsequently diagnosed as having AMD.

Sources of controls

1) Acquaintances of the cases – Patients were asked to introduce (as potential controls) friends of the same sex, within 5 years of their age, who were not known to have AMD.
2) Alternatively, patient's spouses were used as controls if they were over 55 years of age.

3) Controls over 55 years of age were recruited from the outpatient department of the Sydney Eye Hospital.

Cases and controls were postsratified by age (5 year intervals) and sex. Once identified, an appointment was made for each study subject. They were instructed to fast for a minimum of 12 hours. 10 mls of blood were taken at the appointment. A medical history was obtained as well as social and demographic data. All patients then had an ocular examination with pupillary dilation including standard Snellen acuity, applanation tonometry, slit lamp examination of the anterior and posterior segments and fundus examination supplemented with Zeiss fundus photography. The classification of study subjects in terms of macular findings was carried out subsequently using the following criteria:

Cases

1) Patients with pigmentary disturbance only.
2) Patients with drusen. This group is defined as: (a) Patients with Grade 2 AMD defined in the study of Bressler et al. [25], i.e., $\geq$20 small drusen within 1500 um of the foveal centre, or: (b) Any patients with large or confluent or soft drusen or drusen in the presence of pigmentary disturbance.
3) Patients with geographic atrophy. That is, those with areas of well-demarcated atrophy of the retinal pigment epithelium.
4) Patients with neovascular macular degeneration. This group included patients with choroidal neovascularization, haemorrhagic detachments of the RPE and fibrovascular disciform scarring.

The form of AMD was documented for each eye. Where more than one type of degeneration was detected in the two eyes, the most dominant feature (in the order: neovascular > atrophic > drusen > pigmentary disturbance) was used for final classification.

Controls. Controls are defined as individuals without evident macular pathology or with small drusen (less severe than Grade 1 as defined in Bressler et al. [25]), i.e., fewer than 5 small drusen within 1500 μm of the foveal centre and fewer than 10 small drusen between 1500 and 3000 μm from the foveal centre.

Visual acuity. Visual acuity was not a consideration in the selection of cases or controls. However, most controls had pinhole visual acuities 6/9 or better. Those with visual acuities worse than 6/9 in either eye were included when the visual loss could be accounted for by non retinal pathologies. Patients with cataracts that impaired significantly the visualization of their fundi were excluded from the study.

Information obtained. These included demographic details, ocular, medical and "dietary" information and serum analysis.

Demographic details were obtained as follows:
1) Race: Anglosaxon, Eastern European, Southern European and Asian.
2) Education: primary, secondary or tertiary.
3) Marital status: single, married/defacto, divorced or widowed.
4) Height
5) Weight

Ocular factors
1) Iris colour was classified as (a) light or blue pigment, (b) medium or any mixture of brown, grey, green and/or yellow pigments, or (c) dark or brown.
2) Cataract definition as suggested by Jacques et al. [26] was adopted, 'Early lens changes such as flecks, single vacuoles and isolated lamellar separations were not considered to be cataracts. Cortical or subcapsular opacification was considered a cataract only if it was visible against the red reflex and if it consisted of clustered vacuoles, cortical flecks organized in a linear array (minispoke), or more advanced changes. An increase in nuclear opalescence was used as the criterion for the presence of nuclear cataract. This was defined as that which interfered slightly with the view of the detail of the posterior subcapsular area'. The patients were classified into 2 overall cataract categories – absent or present (in either eye).
3) Intraocular pressure (IOP) was measured on the Goldman applanation tonometer for each eye. The values for eyes on glaucoma treatment were not included in the statistical analysis.

Medical factors
1) Blood pressure – systolic (SBP) and diastolic (DBP)
2) Hypertension – including patients with history of hypertension or use of antihypertensives or any one with DBP $\geq$ 95 and SBP $\geq$ 160.
3) Vascular disease (history of) – This was classified as cerebrovascular, cardiovascular, peripheral vascular or 2 or more of the above.
4) Diabetes (history of)
5) Respiratory disease (history of) – This was classified as asthma, chronic airflow limitation or others.
6) Gastrointestinal disease (history of) – This was classified as liver disease, peptic ulceration, intestinal or others.
7) Cancer (history of) – Patients were classified into absent or present in terms of history of any cancer with likely systemic effects.

Dietary factors. Variables which could potentially influence the serum vitamin E or selenium levels or the risk of AMD were ascertained. Smoking and alcohol also fall under this group.
1) Use of wheatgerm.
2) Use of fish liver oil.
3) Use of vitamin E – This was classified as use of multivitamins (containing vitamin E) or use of vitamin E pills.

4) Low fat diet – Patients were asked whether they were on a prescribed low fat diet.
5) Smoking – A detailed smoking history was obtained including current smoking status, current cigarette consumption, an estimate of the total lifetime cigarette consumption and the number of years of abstinence (if applicable).
6) Alcohol – A detailed alcohol history was obtained including current drinking status, current alcohol consumption, an estimate of the total lifetime alcohol consumption and the number of years of abstinence (if applicable).

Serum analysis. Collected blood was allowed to clot at room temperature and then centrifuged. The serum was extracted, stored at −20°C and thawed at the time of analysis. All tubes containing blood or sera were wrapped with tin foil to ensure that the specimens were not exposed to light at any stage of the analysis. The sera were assayed for vitamin E (high pressure liquid chromatography), selenium (flameless atomic absorption spectrometry) and lipids (cholesterol and triglycerides). Lipid assay was necessary due to the fact that serum vitamin E levels are highly correlated with lipid levels including cholesterol levels [27, 28].

Whereas selenium, being a heavy metal, is stable to storage and handling, vitamin E is not. Extreme care was taken in the assay of vitamin E such as the prevention of light exposure and that multiple freezings and thawings were avoided. No specimen was stored for more than 3 months at −20°C. Measurement bias was reduced by having each of the vitamin E and selenium assays performed by one person with no knowledge of the case-control status of the samples.

Statistical methods. The cases and controls were post-stratified by age (5 year intervals) and sex. This increases the likelihood that within each age-sex subgroup, any difference between cases and controls can be due only to some other factor. The statistical group packages GLIM 3.77 and SPIDA were used to analyse the data. The patients' initial characteristics in the case and control groups were compared using t-tests for continuous variables and chi-squared tests for categorical variables. Multivariate logistic regression (adjusted for age and sex) was used to examine the association between AMD and the variables under study.

Classification of study groups

There were 166 individuals in the final study (80 patients and 86 controls). The number of patients from each of the sources described in the previous section is as follows:

Cases:	Source 1 (Followup patients from previous study)	45
	Source 2 (Outpatients)	33
	Source 3 (Controls who became cases)	2

Controls:	Source 1 (Aquaintances of cases)	31
	Source 2 (Spouses of cases)	6
	Source 3 (Outpatient controls)	42

The control group (N = 86) comprised 24 males and 62 females with an age range of 59–86 years (mean, 72); the cases (N = 80) comprised 29 males and 51 females ranging in age from 58–89 years (mean, 76). There was a lower average age of both males and females in the control group compared with cases (controls – average age males 72 years, females 73 years; case – males 75 years, females 76 years). Overall, 53 males and 113 females were studied. Among the cases, there were 5 cases of pigmentary disturbance, 23 of drusen, 18 of geographic atrophy and 34 of neovascular form. There were 3 patients with AMD in one eye only. The distribution of the various types of AMD in each eye was as follows:

	Pigmentary disturbance	Drusen	GA	Disciform
Right eye	6	27	19	27
Left eye	10	28	14	26

The study population was predominantly Anglosaxon (144 out of a total of 166 subjects). There was no significant difference between cases and controls in terms of race, height or weight ($p > 0.1$).

Results

The factors studied are set out below. They are illustrated further in Tables 1–6. For categorical variables, the number of patients in each group is given (not the percentage); for continuous variables, the mean value for each of the case and control groups is given with the standard deviation (SD) shown in brackets.

Serum analysis. Table 1 shows a comparison of the serum data between cases and controls. The mean values are given with the standard deviations

Table 1. Serum analysis of cases and controls (mean, SD in brackets)

	Controls	Cases	*p*-value
Vitamin E (mg/l)	23.7 (7.7)	22.7 (8.5)	>0.1
Cholesterol (mmol/l)	6.1 (1.1)	6.2 (1.1)	>0.1
Triglycerides (mmol/l)	1.6 (0.7)	1.5 (0.7)	>0.1
Selenium (μmol/l)	1.14 (0.19)	1.07 (0.2)	0.02

shown in brackets. There was no significant difference between the two groups with respect to serum vitamin E, cholesterol or triglycerides levels. However, serum selenium was significantly different between the two groups ($p = 0.02$), with the cases having a lower level.

Ocular factors. Table 2 shows a comparison of the ocular factors. There was no significant difference between cases and controls in terms of iris colour, cataract or intraocular pressure.

Medical factors. The medical factors studied included blood pressure and hypertension, vascular disease, diabetes, respiratory disease, gastrointestinal disease and cancer. The results of the first 3 factors mentioned above are shown in Table 3. There were very few patients with the other medical

Table 2. Ocular factors of cases and controls

		Controls	Cases	*p*-value
Categorical factors (numbers shown)				
Iris colour	Light	39	40	
	Medium	30	17	>0.1
	Dark	12	19	
	Unknown	5	4	
Cataract	Absent	40	35	
	Present	45	43	>0.1
	Unknown	1	2	
	Control	Case		p-value
Continuous factors (mean, SD in brackets)				
(R)IOP	15.6 (2.8)	16.4 (3.6)		>0.1
(L)IOP	15.8 (2.9)	16.7 (3.8)		>0.1

Table 3. Medical factors of cases and controls

Variable	Control	Case	*p*-value	
Categorical factors (numbers shown)				
Hypertension	Absent	67	57	>0.1
	Present	19	22	
Vascular	Absent	59	49	
	Cerebro-	5	9	
	Cardio-	17	18	>0.1
	Peripheral	3	2	
	2 of above	2	1	
	Unknown	–	1	
Continuous factors mean, SD in brackets				
Systolic BP		143 (23)	152 (21)	>0.1
Diastolic BP		83 (11)	84 (13)	>0.1

conditions. There was no significant difference between cases and controls in any of the above medical factors.

'*Dietary*' *factors*. Table 4 shows the results of the dietary factors studied. There was no significant difference in vitamin E supplementation either in direct form or in the form of wheatgerm or fishliver oil between cases and controls. Neither did alcohol consumption show any difference. Smokers, however, were significantly more common amongst cases compared to controls ($p < 0.05$). There was also a difference approaching significances ($p = 0.08$) of the number of years of abstinence from smoking (among

Table 4. 'Dietary' factors of cases and controls

		Control	Case	*p*-value
Continuous factors (numbers shown)				
Wheatgerm	No	83	77	
	Yes	3	2	>0.1
	Unknown	–	1	
Fishliver oil	No	83	72	
	Yes	3	7	0.1
	Unknown	–	1	
Vitamin E	No	76	73	
	Multivit.	5	6	>0.1
	Vit. E	4	–	
	Unknown	1	1	
Low fat diet	No	72	70	
	Yes	14	9	>0.1
	Unknown	–	1	
Smoking	Nil	80	67	
(Current)	<1 pack/day	4	11	<0.05
	>1 pack/day	2	2	
Alcohol	Nil	55	51	
(Current)	<20 g/day	26	26	>0.1
	20–40 g/day	1	1	
	>40 g/day	4	2	
Continuous factors mean and SD in brackets				
Current smoking (packs/day)		0.06 (0.24)	0.09 (0.26)	>0.1
Total smoking (pack years)		11.7 (24.9)	12 (18.6)	>0.1
Years of abstinence (among ex-smokers)		13.1 (12.1)	8.6 (8)	0.08
Current alcohol (nips/day)		0.58 (1.26)	0.5 (1)	>0.1
Total alcohol (nip years)		31.3 (70.5)	35.2 (80.5)	>0.1
Years of abstinence (among ex-drinkers)		0 (0)	1.8 (6.4)	>0.1

ex-smokers) between cases and controls. Controls had a longer average period of abstinence than cases. However, neither the current nor total cigarette consumption showed any significant difference between the two groups.

Univariate logistic regression analysis. Table 5 shows the results of univariate logistic regression analysis adjusted for age and sex. The factors with p-values ≤ 0.05 were the same as those from earlier analyses – selenium, smoking status and years of abstinence from smoking, in other words, the significant variables were selenium and smoking. Serum vitamin E level remained insignificant.

Multivariate logistic regression analysis. On multivariate logistic regression analysis of the two variables of interest from univariate analysis (smoking

Table 5. Univariate logistic regression analysis (adjusted for age and sex)

Variable	Odds ratio	95% confidence	p-value
Height	4.8	0.1–171.1	0.4
Weight	0.3	0–5.6	0.4
Cataract	0.9	0.5–1.7	0.7
IOP(R)	1.1	1–1.2	0.3
IOP(L)	1.1	1–1.2	0.3
Hypertension	1.1	0.5–2.3	0.8
Systolic BP	1	0.98–1.01	0.9
Diastolic BP	1	0.98–1.04	0.6
Diabetes	6.4	0.7–57.3	0.1
Cancer	0.8	0.3–2.4	0.7
Wheatgerm use	0.9	0.1–5.8	0.9
Fishoil use	3.4	0.8–14.5	0.09
Vitamin E use	0.6	0.2–1.4	0.2
Low fat diet	0.6	0.3–1.6	0.4
Vitamin E (level)	1	0.95–1.03	0.7
Cholesterol	1.1	0.8–1.4	0.7
Triglycerides	0.9	0.6–1.4	0.6
Selenium	0.2	0.03–1	0.05
Smoking status (smokers vs. non-smokers)	3.2	1.1–9.3	0.04
Total smoking	1	0.96–1	>0.1
Years abstained smoking	0.89	0.78–1	0.05
Alcohol status (drinkers vs. non-drinkers)	1.1	0.6–2.2	>0.1
Total alcohol	1	0.996–1.0053	>0.1
Years abstained alcohol	4	0.001–11100	>0.1

Table 6. Multivariate logistic regression analysis of risk factors

	Odds ratio	95% confidence	*p*-value
Smoking	2.8	0.9–8.2	0.07
Selenium	0.2	0.03–1.17	0.07

and selenium), they both became of borderline significance, each with a *p*-value of 0.07. There was no significant interaction between smoking and selenium.

Comment

There is no universal definition of AMD, the use of decreased visual acuity as a criterion in diagnosis such as in the Framingham Eye Study [29] is no longer used, due to the recognition that early AMD may not impair visual acuity. In the present study, defining cases as having at least 'Grade 2' AMD [25] reduced the likelihood that normal subjects would be misclassified as cases. Defining controls as those falling outside the minimum criterion for 'Grade 1' AMD reduced the likelihood that cases would be misclassified as controls. The study population was predominantly Anglosaxon and this is almost certainly related to the traditionally high participation rate of this group in research studies. There were more females than males in the study (113 vs. 53) and this might be related to the longer life expectancy of females compared to males. The use of fasting blood levels reduces the likelihood of bias in measurement due to any immediate dietary influence and the fact that serum vitamin E level was found to correlate well with the use of vitamin E supplements and cholesterol lends credibility to the validity of the assay. Extensive details of demographic, ocular and medical factors were obtained to minimise the effect of any potential confounder.

In contrast with the Framingham Eye Study [30], height was found not to be associated with AMD. Iris colour was not significant and this is in line with recent findings by Vinding [31]. Cataract was found not to be associated with AMD, however, this result may have been influenced by the exclusion of cases of more advanced cataracts from the study due to research design. Neither hypertension nor systolic or diastolic blood pressures were associated with AMD, however, the number of subjects in the study may be insufficient for this weak reported association to be detected [32]. Vascular disease was not associated with AMD, in contrast with studies which have found a relation [33, 34]. Previous studies on the relationship of smoking and AMD were inconsistent [30, 34, 35]. In the present study, smoking status (smoker vs. non-smoker) correlated with AMD on univariate logistic regression analysis ($p = 0.04$). However, on multivariate analysis, the association was borderline ($p = 0.07$). A smoker may be at a higher risk of AMD than a non-smoker. There was no dose-response effect. Current

cigarette consumption and total cigarette consumption were not associated with AMD. However, among ex-smokers, the number of years of abstinence had a significant association ($p = 0.05$). This seems to be consistent with the above observation that current smoking status correlated with AMD. If this relationship were proven and the relationship between hypertension [32], vascular disease [33, 34] and AMD confirmed, the possibility that changes in the ocular circulation may contribute to the pathogenesis of AMD should be considered.

The result of the present study is in line with that of Blumenkranz et al. [15] who found no relationship between choroidal neovascularization and serum vitamin E levels (based on 26 patients and 23 controls). The present study (based on 80 patients and 86 controls) not only confirmed the above but also found no relationship between serum vitamin E level and AMD. Serum selenium level was found to be of borderline significance ($p = 0.07$). The odds ratio was 0.2, in other words, the risk of AMD may decrease with increasing selenium level. Despite the lower serum selenium levels in the control group, very few were selenium-deficient (only 6 patients with levels below 0.75 μmol/l, the lower range of normal). Furthermore, the difference in selenium levels between cases and controls is small (0.07 μmol/l). Hence the implication of this result is unclear and further studies would be required to clarify the role of selenium in AMD.

While this study does not examine directly the efficacy of antioxidants in the treatment of AMD, the lack of a pronounced association between AMD and serum vitamin E or selenium levels does not support their use. Both vitamin E and selenium are transported in the bloodstream by lipoproteins [22, 36] and the relationship between them, especially in terms of tissue availability, needs further investigations. Tissue uptake and utilization of antioxidants may be defective and achieving high serum antioxidant levels may prove futile. It is known, for instance, that vitamin E uptake in tissues is in association with the receptor-mediated uptake of low density lipoproteins [37]. Since the eye is a small organ, it may be more relevant to perform studies investigating oxidative damage and antioxidant effects locally rather than systemically such as a recent study showing decreased catalase activity in eyes with AMD [38]. Our previous studies have shown that the pathogenesis of AMD is accompanied by a low grade chronic inflammatory response [39, 40] and that leucocytes are related to subretinal neovascularisation [41]. Inflammatory cells have been shown to generate oxygen radicals [42] and if local oxidative damage is related to the pathogenesis of AMD these cells may be one of the contributory factors.

Conclusion

This study found no association between AMD and serum vitamin E level. There was a borderline association between AMD and serum selenium level

using univariate logistic regression analysis, which persisted after adjusting for smoking using multivariate analysis. Nevertheless, the difference in selenium levels between cases and controls was small (0.07 μmol/l).

Thus it is concluded that if oxidative damage is important in AMD, it is not reflected in serum levels of Vitamin E. Further studies are required to clarify the significance of differing serum selenium levels between controls and patients with AMD.

Acknowledgements

The authors are grateful to Mr Athel Turner for advice concerning serum assays, to Dr Karen Wilson for the statistical analyses and to Dr J.M. Provis for reading the manuscript.

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