

Plasma malondialdehyde and nitric oxide levels in age related macular degeneration

Yüksel Totan, Osman Çekiç, Mehmet Borazan, Efkân Uz, Sadık Söğüt, Ömer Akyol

Abstract

Aims—To evaluate alteration of plasma malondialdehyde (MDA) and nitric oxide (NO) levels in patients with exudative age related macular degeneration (ARMD).

Methods—Plasma nitrite plus nitrate concentrations as an index of plasma NO levels and plasma MDA level as a marker of lipid peroxidation were measured in patients with exudative ARMD and age and sex matched healthy subjects.

Results—Significantly higher MDA and lower NO levels were detected in plasma of patients with ARMD compared with their controls ($p=0.01$, $p=0.001$, respectively).

Conclusion—The results may support involvement of oxidative damage and vascular theory in the pathogenesis of ARMD as part of the ageing process.

(*Br J Ophthalmol* 2001;85:1426–1428)

Age related macular degeneration (ARMD) is a multifactorial disease of ageing for which several theories of pathogenesis have been proposed including oxidative damage^{1,2} and ocular perfusion abnormalities.^{3,4}

During ageing, the balance between the generation of reactive oxygen species (ROS) and ROS clearance can be disturbed resulting in oxidative damage to macromolecules such as membrane phospholipids.^{5,6} Within the eye, these damaging reactions have been proposed to be involved in the pathogenesis of ARMD.^{2,7}

Evidence also suggests impaired choroidal blood flow in ARMD.^{3,4} Nitric oxide (NO) modulating vascular tone has an important role in regulation of both systemic and ocular blood flow.^{8,9}

We therefore attempted to determine alterations in the levels of plasma nitrite plus nitrate, the end products of NO, and malondialdehyde (MDA) as an index of lipid peroxidation in patients with ARMD. No report has been found regarding plasma MDA and NO levels in ARMD.

Patients and methods

Patients with exudative ARMD in at least one eye attending the retina service of Turgut Özal Medical Centre were selected for this study. Age and sex matched individuals without ARMD served as controls. After obtaining detailed ophthalmic and medical history, complete ophthalmological examination including slit lamp biomicroscopy of anterior segment, applanation tonometry, funduscopic examination, and fundus fluorescein angiography were performed in all subjects. Exclusion criteria included presence of visually compromising

eye disease such as visually significant cataract, glaucoma, and other retinal diseases. Fasting venous blood samples at the time of ophthalmic examination were obtained, immediately centrifuged, and stored at -70°C until biochemical analysis. The participants were instructed to refrain from drinking beverages containing alcohol or caffeine, or smoking for 24 hours before blood sampling to minimise the contribution to plasma nitrogen oxide levels.

Serum MDA levels were determined using the method described by Wasowicz *et al.*¹⁰ Briefly, MDA was reacted with thiobarbituric acid by incubating for 1 hour at $95\text{--}100^{\circ}\text{C}$. Following the reaction, fluorescence intensity was measured in the *n*-butanol phase with a fluorescence spectrophotometry (Hitachi, Model F-4010) (excitation at 525 nm, emission at 547 nm). Results were expressed as $\mu\text{mol/l}$.

Plasma nitrite plus nitrate concentrations as an index of plasma NO levels, were determined by the method described previously.¹¹ Quantification of nitrite and nitrate was based on the Griess reaction, in which a chromophore with a strong absorbance at 540 nm is formed by reaction of nitrite with a mixture of naphthylethylenediamine and sulphanilamide. The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biochrom Ltd, Cambridge, UK) to give the nitrite concentration. For nitrate detection, a second sample was treated with copperised cadmium in glycine buffer at pH 9.7 to reduce nitrate to nitrite, the concentration of which thus represented the total nitrite plus nitrate. A standard curve was established with a set of serial dilutions ($10^{-8}\text{--}10^{-3}$ mol/l) of sodium nitrite. All samples were assayed in duplicate. Results were expressed as $\mu\text{mol/l}$.

All statistical analyses were performed using SPSS statistical software (SPSS for Windows, Version 7.0, CA, USA).

Results

The mean age was 68 years in ARMD group (14 female, six male) and 65.9 years in the control group (seven female, three male). Smoking history was noted in four subjects in the ARMD group and three in the control group. The number of subjects with systemic hypertension was two in the study group and three in the control group. There were no other associated systemic diseases like diabetes and antioxidant vitamin (that is, vitamin C and E) use, which may interact with the production of MDA and/or NO in both groups.^{12–14}

Figures 1 and 2 show scatter plots of the groups versus serum MDA and nitrite plus

Department of
Ophthalmology,
Turgut Özal Medical
Centre, İnönü
University School of
Medicine, Malatya,
Turkey
Y Totan
O Çekiç
M Borazan

Department of
Biochemistry
E Uz
S Söğüt
Ö Akyol

Correspondence to:
Dr Yüksel Totan, Hastane
caddesi No 44/7, 44300
Malatya, Turkey
ytotan@usa.net

Accepted for publication
15 June 2001

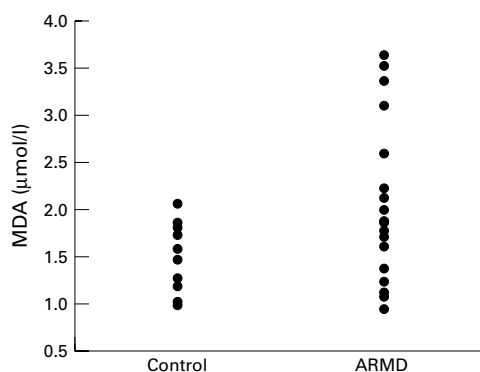


Figure 1 Plot of plasma malondialdehyde (MDA) levels in patients with age related macular degeneration (ARMD) and control subjects.

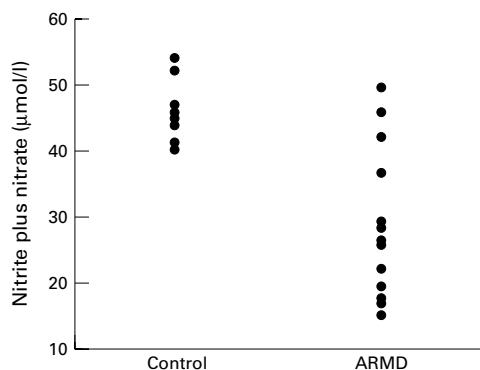


Figure 2 Plot of plasma nitrite plus nitrate levels in patients with age related macular degeneration (ARMD) and control subjects.

nitrate levels. The mean (SEM) serum MDA level was 2.18 (0.19) $\mu\text{mol/l}$ in patients with exudative ARMD and 1.53 (0.11) $\mu\text{mol/l}$ in the controls (median values were 2.06 $\mu\text{mol/l}$ and 1.56 $\mu\text{mol/l}$ respectively). The difference was statistically significant ($p=0.01$). Serum nitrite plus nitrate levels were significantly lower in ARMD group (mean 27.34 (2.73) $\mu\text{mol/l}$; median 25.65 $\mu\text{mol/l}$) compared to the control group (mean 45.38 (1.51) $\mu\text{mol/l}$; median 44.75 $\mu\text{mol/l}$) ($p=0.001$).

Discussion

Plasma thiobarbituric acid reacting substances increase with age, indicating increased lipid peroxidation.¹⁵ In this preliminary study, the result of increased lipid peroxidation in serum samples from ARMD patients is consistent with those of previous studies^{16 17} in which age related decreases in the capacity of antioxidant systems have been found to be more pronounced in patients with ARMD compared with controls. High polyunsaturated fatty acid content of photoreceptor membranes particularly expose the retina to increased risk of lipid peroxidation by unopposed action of free radicals.¹⁸ It has been suggested that the retina is very susceptible to lipid peroxidation,^{7 19} and that this susceptibility also increases with ageing in the macular region.⁷

The plasma level of MDA, a byproduct of lipid peroxidation, is a reliable and commonly used biomarker of the overall lipid peroxidation. Our finding of increased plasma MDA

levels in ARMD patients is not only consistent with the role of oxidative stress in ARMD, but also supports the idea that plasma MDA levels may be used as a marker of oxidative stress on a group basis.²⁰

The vascular theory of ARMD involves primarily the choroidal perfusion defects which have been identified in both non-exudative and exudative forms using fluorescein and indocyanine angiographic methods, laser Doppler flowmetry, and colour Doppler imaging, and could account for some of the pathological changes in ARMD, since the choriocapillaris supplies the metabolic needs of the retinal pigment epithelium.⁴ NO has a regulatory role in ocular as well as systemic blood flow.^{8 9} Systemic NOS inhibition was shown to decrease basal choroidal blood flow in healthy subjects,^{21 22} and administration of systemic NO precursors has been found to increase ocular²³ and, specifically choroidal, blood flow.²⁴ Colour Doppler studies demonstrated retrobulbar blood flow abnormalities including impaired choroidal blood flow in exudative and non-exudative ARMD.^{3 25} Evidence also suggested the protective role of NO against hypertrophy of resistance blood vessels²⁶ and atherosclerosis,²⁷ which is why reduced plasma NO level in our ARMD patients may be closely related to the decrease in the compliance of the choroidal vessels postulated by Friedman's haemodynamic model of pathogenesis of ARMD.²⁸ Therefore, decreased plasma nitrite plus nitrate concentration, an index of the plasma NO level, in our patients with exudative ARMD is consistent with the vascular theory of ARMD.

Nitric oxide is a free radical with an unpaired electron allowing it to reduce other molecules. Therefore, NO may act as a potential antioxidant agent and inhibit lipid peroxidation. However, physiological actions of NO are destroyed by the superoxide radical and stabilised by superoxide dismutase which catalyses the breakdown of superoxide radical. The short lived NO and mildly reactive superoxide radical rapidly combine to form a potent and long lived oxidant, peroxynitrite which then breaks down to form a hydroxyl radical, thereby resulting in increased lipid peroxidation. Thus, in conditions of increased oxidative stress, excess superoxide radical decreasing NO bioavailability through peroxynitrite formation may inhibit the regulatory effects of NO on systemic and ocular blood flow. Conversely, the administration of antioxidants may effectively enhance NO levels by decreasing the availability of free radical species.^{29 30} We have found a decreased plasma NO level with increased lipid peroxidation in ARMD patients compared to their controls. Besides a decreased plasma NO level, the interaction between NO and other free radicals, particularly superoxide, also seems to increase the risk of choroidal perfusion defect as well as lipid peroxidation, the two important mechanisms for development of ARMD.

Regarding the decreased plasma NO level in the ARMD patients, one possible explanation might be increased free radical production with defective antioxidant defence mechanisms in

ARMD, which may directly induce endothelial cell damage, and therefore cause decreased production of NO by endothelial nitric oxide synthase (eNOS). However, we could not clarify, in this preliminary study, the causative factors for the reduction of the plasma NO level, nor whether this alteration was a predisposing factor or a result of ARMD.

The results of this preliminary study suggest that the possible alterations of plasma MDA and NO levels are associated with ARMD, but requires further studies to evaluate the related mechanisms and their interactions leading to the biochemical changes in this disorder.

- 1 Delcourt C, Cristol JP, Leger CL, et al. Associations of antioxidant enzymes with cataract and age-related macular degeneration. The POLA Study. *Ophthalmology* 1999;106:215–22.
- 2 Cai J, Nelson KC, Wu M, et al. Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 2000;19:205–21.
- 3 Friedman E, Krupsky S, Lane AM. Ocular blood flow velocity in age-related macular degeneration. *Ophthalmology* 1995;102:640–6.
- 4 Harris A, Chung HS, Ciulla TA, et al. Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog Retin Eye Res* 1999;18:669–87.
- 5 Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidant, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 1993;90:7915–22.
- 6 Harman D. Aging: phenomena and theories. *Ann NY Acad Sci* 1998;854:1–7.
- 7 De La Paz MA, Anderson RE. Regional and age-dependent variation in susceptibility of the human retina to lipid peroxidation. *Invest Ophthalmol Vis Sci* 1992;33:3497–9.
- 8 Koss MC. Functional role of nitric oxide in regulation of ocular blood flow. *Eur J Pharmacol* 1999;374:161–74.
- 9 Stamler JS, Loh E, Roddy M, et al. Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation* 1994;89:2035–40.
- 10 Wasowicz W, Neve J, Peretz A. Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage. *Clin Chem* 1993;39:2522–6.
- 11 Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem* 1990;36:1440–43.
- 12 Gallou G, Ruelland A, Legras B, et al. Plasma malondialdehyde in type 1 and type 2 diabetic patients. *Clin Chim Acta* 1993;214:227–34.
- 13 Honing MLH, Morrison PJ, Banga JD, et al. Nitric oxide availability in diabetes mellitus. *Diabetes Metab Rev* 1998;14:241–9.
- 14 Brown KM, Morrice PC, Duthie GG. Vitamin E suppresses indexes of lipid peroxidation and platelet count in blood of smokers and nonsmokers but plasma lipoprotein concentrations remain unchanged. *Am J Clin Nutr* 1994;60:383–7.
- 15 Coudray C, Roussel AM, Arnaud J, et al. Selenium and antioxidant vitamin and lipid peroxidation levels in pre-aging French population. EVA Study Group. *Biol Trace Elem Res* 1997;57:183–90.
- 16 Prashar S, Pandav SS, Gupta A, et al. Antioxidant enzymes in RBCs as a biological index of age related macular degeneration. *Acta Ophthalmol* 1993;71:214–18.
- 17 Cohen SM, Olin KL, Feuer WJ, et al. Low glutathione reductase and peroxidase activity in age-related macular degeneration. *Br J Ophthalmol* 1994;78:791–4.
- 18 Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. In: Holman RT, ed. *Progress in lipids research*. Vol 22. Elmsford, NY: Pergamon Press, 1983:79–131.
- 19 Ito T, Nakano M, Yamamoto Y, et al. Hemoglobin-induced lipid peroxidation in the retina: a possible mechanism for macular degeneration. *Arch Biochem Biophys* 1995;316:864–72.
- 20 Nielsen F, Mikkelsen BB, Nielsen JB, et al. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem* 1997;43:1209–14.
- 21 Schmetterer L, Krejcy K, Kastner J, et al. The effect of systemic nitric oxide-synthase inhibition on ocular fundus pulsations in man. *Exp Eye Res* 1997;64:305–12.
- 22 Luksch A, Polak K, Beier C, et al. Effects of systemic NO synthase inhibition on choroidal and optic nerve head blood flow in healthy subjects. *Invest Ophthalmol Vis Sci* 2000;41:3080–4.
- 23 Liu SXL, Chen Z, Xuan B, et al. Effects of S-nitrosoglutathione and 4-phenyl-3-fluoroxycarbonitrile on ocular blood flow and retinal functions through generation of nitric oxide. *J Ocular Pharmacol Ther* 1997;13:105–14.
- 24 Xuan B, Zhou YH, Varma R, et al. Effects of some N-nitroimidazole derivatives on ocular blood flow and retinal function recovery after ischemic insult. *J Ocular Pharmacol Ther* 1999;15:135–42.
- 25 Ciulla TA, Harris A, Chung HS, et al. Color Doppler imaging discloses reduced ocular blood flow velocities in nonexudative age-related macular degeneration. *Am J Ophthalmol* 1999;128:75–80.
- 26 Deng LY, Thibault G, Schiffrin EL. Effect of hypertension induced by nitric oxide synthase inhibition on structure and function of resistance arteries of the rat. *Clin Exp Hypertens* 1993;15:527–37.
- 27 Vane JR, Botting RM. Impact of risk factors on the endothelium. *Cardiovasc Risk Factors* 1994;4:108–21.
- 28 Friedman E. A hemodynamic model of the pathogenesis of age-related macular degeneration. *Am J Ophthalmol* 1997;124:677–82.
- 29 Violi F, Marino R, Milite MT, et al. Nitric oxide and its role in lipid peroxidation. *Diabetes Metab Res Rev* 1999;15:283–88.
- 30 Goldstein IM, Ostwald P, Roth S. Nitric oxide: a review of its role in retinal function and disease. *Vis Res* 1996;36:2979–94.



Plasma malondialdehyde and nitric oxide levels in age related macular degeneration

Yüksel Totan, Osman Çekiç, Mehmet Borazan, et al.

Br J Ophthalmol 2001 85: 1426-1428

doi: 10.1136/bjo.85.12.1426

Updated information and services can be found at:

<http://bjo.bmj.com/content/85/12/1426.full.html>

References

These include:

This article cites 27 articles, 9 of which can be accessed free at:

<http://bjo.bmj.com/content/85/12/1426.full.html#ref-list-1>

Article cited in:

<http://bjo.bmj.com/content/85/12/1426.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

[Retina](#) (1217 articles)

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>