Plasma malondialdehyde and nitric oxide levels in age related macular degeneration

Yüksel Totan, Osman Çekiç, Mehmet Borazan, Efkan 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.

Age related macular degeneration (ARMD) is a multifactorial disease of ageing for which several theories of pathogenesis have been proposed including oxidative damage and ocular perfusion abnormalities.

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. Within the eye, these damaging reactions have been proposed to be involved in the pathogenesis of ARMD.

Evidence also suggests impaired choroidal blood flow in ARMD. Nitric oxide (NO) modulating vascular tone has an important role in regulation of both systemic and ocular blood flow.

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, ophtalmodynamometry, funduscopy 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. Briefly, MDA was reacted with thiobarbituric acid by incubating for 1 hour at 95–100°C. Following the reaction, fluorescence intensity was measured in the n-butanol phase with a fluorescence spectrophotometer (Hitachi, Model F-4010) (excitation at 525 nm, emission at 547 nm). Results were expressed as μmol/l.

Plasma nitrite plus nitrate concentrations as an index of plasma NO levels, were determined by the method described previously. 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 naphthylethenediamine and sulphanilamide. The absorbance was measured in a spectrophotometer (Ultraspec Plus, Pharmacia LKB Biocrom Ltd, Cambridge, UK) to give the nitrite concentration. For nitrate detection, a second sample was treated with cupperised 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 (10⁻⁴–10⁻³ mol/l) of sodium nitrite. All samples were assayed in duplicate. Results were expressed as μmol/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.

Figures 1 and 2 show scatter plots of the groups versus serum MDA and nitrite plus...
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Peroxidation. It has been suggested that the retina has 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 involved in the vascular theory of ARMD.28 The short lived NO and mildly reactive superoxide radical rapidly combine to form a potent and long lived oxidant, peroxynitrite which catalyses the breakdown of superoxide radical. The short lived NO and mildly reactive superoxide radical might be increased free radical production with increased lipid peroxidation in ARMD. We have found a decreased 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 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.29

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.1 NO has a regulatory role in ocular as well as systemic blood flow.3,4 Systemic NOS inhibition was shown to decrease basal choroidal blood flow in healthy subjects, 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.5,6 Evidence also suggested the protective role of NO against hypertrophy of resistance blood vessels and atherosclerosis, 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 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.29

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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.

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