BIOMARKERS OF OXIDATIVE STRESS IN PATIENTS WITH WET AGE RELATED MACULAR DEGENERATION

P. ZAFRILLA¹, M. LOSADA², A. PEREZ¹, G. CARAVACA¹, J. MULERO²

1. Department of Food Technology and Nutrition. Catholic University of San Antonio. Murcia 30107, (Spain); 2. University Hospital Jose Mª Morales Meseguer.
Corresponding author: P. Zafirilla, Department of Food Technology and Nutrition, Catholic University of San Antonio, Murcia 30107, Spain. Email: mpzafirilla@ucam.edu,
Phone: +34968278751, Fax: +34968278622

Abstract: Objective: The aim of this study was to analyze biomarkers of oxidative stress in patients with wet age related macular degeneration (AMD). Participants and Measurements: Case-control study that includes 163 patients with wet AMD (age group of 55–82 years with the mean age of 71 years and 170 age-matched healthy controls in the age group of 55–78 years with the mean age of 71 years. The following parameters were determined: reduced and oxidized Glutathione (GSH/GSSH), protein carbonyl groups, total antioxidant activity in plasma and the activity of endogenous antioxidant enzymes, such as, glutathione peroxidase, glutathione reductase and superoxide dismutase. Results: We observed total antioxidant activity higher in control group (CG) compared with patients with wet AMD (71 ± 1.2 μM Trolox vs 58 ± 1.1 μM Trolox). Values of superoxide dismutase activity (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) are higher in control group than in patients with wet AMD. According to the GSH/GSSH results, average values were higher in the CG than in patients with wet AMD and data were not significantly different. Values of protein carbonyl groups were higher in patients with wet AMD than in CG and significant differences were found. Conclusions: The finding of the present study suggests that the patients with wet AMD are an altered metabolic state of oxidation-reduction and that it is convenient to give therapeutic interventions with antioxidants. We have demonstrated that systematic oxidative stress, measured by different biomarkers is closely associated with the wet AMD.

Key words: Protein carbonyl groups, GSH/GSSH, age related macular degeneration (ARMD), antioxidant enzymes.

Introduction

Age-related macular degeneration (AMD) is a devastating condition characterized by the deterioration of the macula in which central vision becomes severely impaired. There are two forms of macular degeneration: atrophic (dry) and neovascular (wet). Both forms of the disease may affect both eyes simultaneously. Age-related declines in the retinal carotenoid pigment content, coupled with photo damage induced by harmful Ultraviolet (UV) rays, give rise to this debilitating condition. The progression and severity of macular degeneration, as with all age-related diseases, are exacerbated by factors such as oxidative stress, inflammation, high blood sugar, and poor vascular health. Approximately 85-90 percent of AMD cases are the dry form. Wet AMD, which represents only 10-15 percent of AMD cases, is responsible for more than 80 percent of blindness. AMD is equally common in men and women, and has a heritable nature (1, 2).

Oxidative stress, which refers to cellular damage caused by reactive oxygen intermediates (ROI), results due to disturbed equilibrium between pro oxidants and antioxidants. Oxidative stress has been implicated in many disease processes, especially age-related disorders (3). Retinal factors (intensive oxygen metabolism, continual exposure to light, a high concentration of polyunsaturated fatty acids, the presence of photosensitizers) increase the production of reactive oxygen species. In vitro studies have consistently shown that photochemical retinal injury is attributable to oxidative stress. Furthermore, there is strong evidence suggesting that lipofuscin (a photoreactive substance) is derived, at least in part, from oxidatively damaged photoreceptor outer segments (4). While naturally occurring antioxidants typically manage this, environmental factors and stress can decrease circulating antioxidants. For example, levels of the endogenous antioxidant glutathione decrease as people age, making the lens nucleus and retina susceptible to oxidative stress (5).

Macular pigment is also believed to limit retinal oxidative damage by absorbing incoming blue light and/or quenching ROIs. Many putative risk-factors for AMD have been linked to a lack of macular pigment, including female gender, lens density, tobacco use, light iris color, and reduced visual sensitivity (3).

Vitamin C, normally highly concentrated in the aqueous humor and corneal epithelium, helps absorb damaging ultraviolet radiation, protect the basal layer of the epithelium, and prevent AMD (6). L-carnosine and vitamin E also mitigate oxidative stress and free-radical damage (5).

The aim of this study was to analyze biomarkers of oxidative stress in patients with wet age related macular degeneration (AMD) compared with control group without AMD.

Subjects and Methods

Study Population
A total of 163 patients with wet AMD (age group of 55–82 years with the mean age of 71 years (80 Male and 83 Female)...
Blood sampling

Blood samples were obtained in similar conditions (in fasting) in metabolic syndrome patients and control group. Blood samples were collected from the median cubital vein and placed in EDTA-containing vials. Blood is centrifuged to obtain serum at 3000x g for 15 min at room temperature within 1 h of collection and stores at -80° C until the assays were performed.

Measurement of total antioxidant capacity

Total antioxidant status (TAS) in plasma was measured by ORAC method. The oxygen radical absorbance capacity was determined as described by Dávalos et al. (7) with slight modifications. The ORAC analyses were carried out on a Synergy HT multi-detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, Vt, USA), using 96-well polystyrene microplates with black sides and clear bottom, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by KC4, version 3.4, software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 µL. Fluorescein (FL) (100 µL; 3 nM, final concentration) and serum (70 µL), were placed in the wells of the microplate. The mixture was preincubated for 30 min. at 37 °C, before rapidly adding the 2,2’-azobis(2-aminopropane) dihydrochloride (AAPH) solution (30 µL; 19 mM, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 1.14 min. for 120 min. The microplate was automatically shaken prior to each reading. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution, and eight calibration solutions using Trolox C as antioxidant were also used in each assay. All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 µL of distilled water. The antioxidant abilities were expressed as µM Trolox equivalents.

Measurement of enzymatic activity

For the quantitative determination of glutathione peroxidase in the blood the Ransel commercial kit was used. This method is based on that of Plagia and Valentine (8). Glutathione Peroxidase (GPx) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. Absorbancy reduction was measured at 340 nm for 2 min and the GPx activity was expressed as U/L of sample.

The analysis of erythrocyte superoxide dismutase activity was performed using Randox (Randox, Crumlin, UK). The activities were measured enzymatically at 37°C on a Varian spectrometer (mod. Cary Bio-50 UV-Vis) at 505 nm, as previously reported Delmas-Beauvieux et al. (10). Randox provided standards. SOD activity was expressed as U/g Hb of sample.

Measurement of Protein carbonyl groups

Were determined by an ELISA kit (Biocell Corporation Ltd, New Zealand) according to the manufacturer’s intructions.

Measurement GSH/GSSH

Reduced and oxidized Glutathione were determined by colorimetric determination (OxisResearch TM Bioxytech GSH/GSSH-412 TM Burlingame, USA) according to the manufacturer’s intructions.

Statistical analysis

All data were analyzed by using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL). Descriptive statistic is presented as mean ± standard deviation. Means were compared by the variance test of repeated means. Analysis of variance (ANOVA) was used to examine significant differences in the protein carbonyl groups, GSH/GSSH ratio, superoxide dismutase activity (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) of the two groups of study. A probability of less than 0.05 (p<0.05) was considered statistically significant.

Results

We observed total antioxidant activity higher in CG (control group) compared with patients with wet AMD and significant